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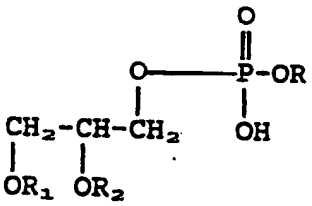

(57) Abstract

The invention relates to a compound having formula (A) or pharmaceutically acceptable salts thereof, wherein one of R_1 and R_2 is a heteroatom fatty acid acyl group having 13-14 carbon atoms in the principal chain and up to a total of 18 carbon atoms, while the other is hydrogen, a heteroatom of fatty acid acyl group containing 13-14 carbon atoms in the principal chain and up to a total of 18 carbon atoms or an acyl group of a fatty acid containing 4-26 carbon atoms in the principal chain and up to a total of 30 carbon atoms and R is a naturally occurring polar group characteristic of a glycerolphospholipid isolated from endogenous sources. This invention also relates to the use of the compounds of the present invention for inhibiting retrovirus infections and for the treatment of AIDS or AIDS related diseases.

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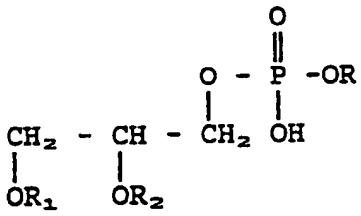
NOVEL ACYLATED PHOSPHOLIPID DRUGS

The present invention relates to novel glycerol phospholipids useful as a drug for the treatment of AIDS and AIDS related complex. More specifically, the present invention is directed to a compound of the formula:



or pharmaceutically acceptable salts wherein one of R_1 and R_2 is a heteroatom fatty acid acyl group containing 13-14 carbon atoms in the principal chain and up to a total of 18 carbon atoms, while the other is hydrogen, a heteroatom fatty acid acyl group containing 13-14 carbon atoms in the principal chain and up to a total of 18 carbon atoms or an acyl group of a fatty acid containing 4-26 carbon atoms in the principal chain and up to a total of 30 carbon atoms and

R is a naturally occurring polar group characteristic of a glycerophospholipid isolated from endogenous sources.

The present compounds are useful in the treatment of AIDS. More specifically, the compounds of the present invention possess anti-viral activity especially anti-retroviral activity. Thus, these compounds of the present invention are useful in combatting and/or retarding the growth of retroviruses, such as the human immunodeficiency virus (HIV). As such, the compounds of the present invention are useful

in treatment of acquired immune deficiency syndrome (AIDS) and AIDS-related complex (ARC).

The effectiveness of the compounds of the present invention are illustrated in the following figures, which are discussed in more detail hereinbelow.

Figure 1 depicts the anti-HIV activity in T-cells of AC1, AC2, 1-(12-methoxydodecanoyl)-sn-3-glycerophosphatidylcholine and 12-methoxydodecanoic acid. Results are plotted on mM scale for direct comparison. Toxicity of the compounds in the CEM cells is labelled above each bar as toxic or non-toxic. The % reduction in the direct cytopathic effect of the virus (CPE) is represented on the Y-axis.

Figure 2 shows the anti-HIV activity in macrophages of L-AC1, L-AC2 and 12MO measured with an HIV p24 antigen assay.

Figure 3 depicts the anti-HIV activity of L-AC2 and 12MO in MT-4 cells using syncytial cell assays.

Figure 4 depicts the antiviral activity of L-AC2 and 12MO in peripheral blood monocytes (PBMC's) measured by reverse transcriptase assay. The results are depicted as the % of control.

Figure 5 demonstrates the toxicity activity of L-AC2 and 12MO used to evaluate the concentration of drug that kills 50% of MT-4 cells in the absence of virus. Dose response curves were used to evaluate the concentration of drug that kills 50% of MT4 cells. This 50% effective toxicity to cells is denoted as TC50 in each graph.

Figure 6 depicts the potent anti-HIV synergism when L-AC2 and AZT are concurrently administered.

Figure 7 depicts the anti-HIV activity of D-AC2 (unnatural glycerophosphatidyl choline configuration) and phosphatidylcholine (PE) analogs, L-PE-1 and L-PE-2 compared with 12MO.

Figure 8 depicts the stability of various anti-HIV phospholipids, L-PE1, L-AC2, D-AC2 and L-PE2 in fresh blood at 38°C.

As used herein, the pharmaceutically acceptable salts include the acid and basic salts. Basic salts for pharmaceutical use are potassium, sodium, calcium, magnesium, zinc and the like. Suitable acids include for example, hydrochloric, sulfuric, nitric, benzenesulfonic, toluenesulfonic, acetic, maleic, tartaric and the like which are pharmaceutically acceptable.

The term "lower alkyl" refers to an alkyl group containing from 1 to 6 carbon atoms and may be straight chain or branched. It includes such groups as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, t-butyl, pentyl, amyl, hexyl and the like. The preferred lower alkyl group is methyl.

The term "fatty acid" shall mean a carboxylic acid derived from or contained in animal or vegetable fat or oil. Said fatty acids may be saturated or unsaturated and are composed of a chain of alkyl groups containing from 4 to 26 carbon atoms, usually even numbered. The fatty acids are characterized by a terminal carboxy group. They also may contain a hydroxy group or a second carboxy group. It is preferred that the second carboxy group, when present, is located at the omega (last) carbon position of the principal chain.

Examples of fatty acids are described in SCIENTIFIC TABLES, 7th Edition, published by CIBA-Geigy Limited, Basle Switzerland, p. 365-372 (1970), and the contents are incorporated by reference as if fully set forth herein. These examples include the natural product fatty acids, such as propionic acid, n-butyric acid, valeric acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid, capric acid, undecylic acid, lauric acid, margaric acid, stearic acid, nondecylic acid, arachidic acid, heneicosanoic acid, behenic acid, tricosonoic acid, lignoceric acid, pentacosanoic acid, cerotic acid, acrylic acid, trans-(α)-crotonic acid, iso(ß)-crotonic acid, Δ^2 hexenoic acid, Δ^4-decenoic acid, Δ^9-dodecanoic acid, Δ^4 dodecanoic acid, Δ^6-dodecanoic acid, tsuzuic acid, physteric acid, myristoleic acid, palmitoleic acid, petroselinic acid, oleic acid, eladic acid, trans- and cis-vaccenic acid, Δ^{12}-octadecenoic acid, gadoleic acid. Δ^{11}-eicosenoic acid, cetoleic acid, erucic acid, brassidic acid, selacholeic acid, ximenic acid, sorbic acid, linoleic acid, hiragonic acid, α-eleosteric acid, ß-eleostearic acid, linolenic acid, stearidonic acid, arachidonic acid, behenolic acid, isobutyric acid, isovaleric acid, tiglic acid, isomyristic acid, anteiomargic acid, tuberculostearic acid, phytanic acid, myocolipenic acid, myococeranic acid, and the like. Preferably, the term fatty acid as used herein shall contain 10 to 22 carbon atoms, and more preferably shall contain 13 to 18 carbon atoms. Most preferably, the fatty acid shall contain 4-8 carbon atoms or 13-15 carbon atoms.

As used herein, the term "fatty acyl of a fatty acid" is defined as a fatty acid in which the carboxy terminus is replaced by an acyl group ($-\overset{\overset{O}{\|}}{C}-$). In other words, said term has the formula $\overset{\overset{O}{\|}}{C}-R_7$, wherein R_7 is a hydrocarbyl group as defined herein. For example, the fatty acyl of myristic acid is

$$CH_3-(CH_2)_{12}-\overset{\overset{O}{\|}}{C}-.$$

It is preferred that the fatty acids acyl group contains 4-26 carbon atoms. Besides the acyl group, $(\overset{\overset{O}{\|}}{C})$, the fatty acid acyl group may contain unsaturation, e.g., double or triple bonds between the carbon atoms, but it is preferred that the fatty acid acyl group contains single bonds between the carbon atoms. In fact, except for the acyl, it is preferred that this group is a hydrocarbyl group, as defined herein. It is especially preferred that the hydrocarbyl group be saturated and contain 4-16 carbon atoms, and most preferably 4-14 carbon atoms. Finally, it is preferred that the group contains an even number of carbon atoms.

The fatty acyl group may be straight chained or branched, but it is preferred that it is straight chained. Examples include $CH_3(CH_2)_2\overset{\overset{O}{\|}}{C}-$, $CH_3(CH_3)_4\overset{\overset{O}{\|}}{C}-$, $CH_3(CH_2)_6\overset{\overset{O}{\|}}{C}-$, $CH_3(CH_2)_8\overset{\overset{O}{\|}}{C}-$,

$CH_3(CH_2)_{10}-\overset{\overset{O}{\|}}{C}-$, $CH_3(CH_2)_2-\overset{\overset{O}{\|}}{C}-$, and the like.

The term "heteroatom fatty acid" is a biologically active fatty acid analog of myristic acid chosen from a saturated or partially unsaturated fatty acid containing 13-14 carbon atoms, wherein at least one methylene group normally present at position 4 to 13 is replaced by at least one oxygen or sulfur atom. It is preferred that the heteroatom fatty acid contain 1-3 oxygen or sulfur atoms or a combination thereof. It is especially preferred that only one methylene group is replaced by oxygen or sulfur and it is most preferred that said methylene group is replaced by oxygen.

Preferred heteroatom fatty acids employable in the present invention include but are not limited to: 11-(ethylthio)undecanoic acid [$CH_3CH_2S(CH_2)_{10}COOH$]; 5-(octylthio)pentanoic acid [$CH_3(CH_2)_7S(CH_2)_4COOH$]; 11-(methoxy)undecanoic acid [$CH_3O(CH_2)_{10}COOH$]; 11-(ethoxy)undecanoic acid [$CH_3CH_2O(CH_2)_{10}COOH$]; 12-(methoxy)dodecanoic acid [$CH_3O(CH_2)_{11}COOH$]; 10-(propylthio)decanoic acid [$CH_3(CH_2)S(CH_2)_9COOH$]; 10-(propoxy)decanoic acid [$CH_3(CH_2)_2O(CH_2)_9COOH$]; 11-(1-butoxy)undecanoic acid [$CH_3(CH_2)_3O(CH_2)_{10}COOH$]; [10-(2-propynoxy) decanoic acid [$HC\equiv CCH_2O(CH_2)_9COOH$]; and the like.

Additionally, the term heteroatom fatty acid is a saturated or unsaturated C_{13} to C_{14} fatty acid which is substituted by halo, hydroxy, alkoxy, mercapto or alkylthio. More preferably, the heteroatom fatty acid is a saturated or unsaturated fatty acid containing 13 to 14 carbon atoms which is substituted with halo or

hydroxy. More preferred heteroatom fatty acids are saturated or unsaturated fatty acids which contain 13 to 14 carbon atoms and are substituted by chloro, bromo or hydroxy. Still more preferred are saturated or unsaturated fatty acids which contain 13 to 14 carbon atoms which are substituted by chloro, bromo or hydroxy at the 2-position.

Additionally, it is to be understood, within the spirit and scope of the present invention, that the term heteroatom fatty acid may also be a C_{13} to C_{14} saturated or unsaturated fatty acid wherein a methylene group normally at carbon position 5 to 12 is replaced by oxygen or sulfur, and further, said fatty acid may be substituted, preferably at the 2-position, by halo, hydroxy, alkoxy, mercapto or alkylthio.

The term "alkyl heteroatom fatty acid acyl group" as defined herein is defined as a heteroatom fatty acid containing no multiple carbon-carbon bonds in which the carboxy terminus is replaced by an acyl group $(-\overset{\overset{O}{\|}}{C}-)$.

As used herein, the term halo shall mean one or more members of Group VII A of the periodic table, including fluorine, chlorine, bromine, and iodine; most preferably, fluoro or chloro and especially bromo.

The term alkoxy denotes an o-alkyl group, wherein alkyl is defined hereinabove. Examples of alkoxy are methoxy, ethoxy, propoxy, butoxy, pentoxy, hexoxy, and the like.

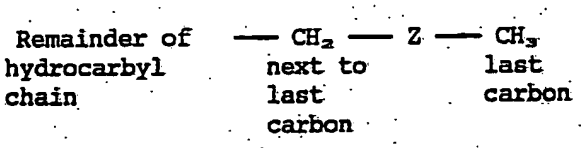
The term alkylthio is defined as an alkyl, as hereinbefore defined, containing a thio group.

The term mercapto shall mean HS.

The term "omega (w) carbon" refers to the last carbon in the principal chain.

The term penultimate carbon refers to the next to last carbon on the principal chain. For example, in the decyl substituent, C_{10} is the omega carbon while C_9 is the penultimate carbon.

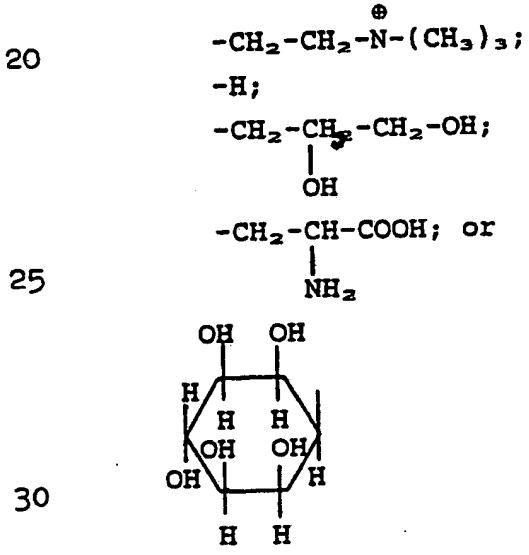
The term "heteroatom is bonded to the penultimate carbon" or any equivalence thereof, means that the heteroatom is bonded between the omega and penultimate carbon. For example, if Z is a heteroatom, and if it is stated that Z is bonded to the penultimate carbon, this means that in the principal chain, Z is located between the last and the next to last carbon:



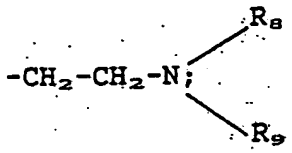
As defined herein, R is part of the polar head group and is a distinguishing portion of a glycerophospholipid. The polar group may be naturally occurring or analogs thereof. There are many types of polar groups on glycerol phospholipids found in nature. The more common R groups thereon are inositol, ethanolamine, choline and serine. But recently other head groups have been found, e.g., N-methyl ethanolamine, N,N-dimethyl ethanolamine, (See Casal et al., Biochemica et Biophysica Acta, 1983, 735, 387-396), and sulfocholine, (See Mantsch et al. Biochemical et Biophysica Acta, 1982, 689, (63-72) and the like. The present invention contemplates these groups found in

naturally occurring glycerophospholipids and analogs thereof.

The polar groups used herein have a dipole moment. These polar groups may contain heteroatoms, such as O, S or N or P. In fact, they may contain more than 1 heteroatom, e.g., 2, 3, 4, 5 or 6 - 9. Thus, there may be as many as 8 or 9 heteroatoms present in the R group. The polar head group may be a natural sugar (e.g., inositol) or combination of natural sugars (e.g., inositol-glycon) or the R group may consist of an alkylene chain in which a methylene group is replaced by a heteroatom or a heteroatom lower alkyl ($N-CH_3$, e.g.) or heteroatom diloweralkyl [e.g. $S(CH_3)_2$], or if the heteroatom is nitrogen, a triloweralkyl heteroatom. Examples include inositol, ethanolamine, choline, sulfocholine, serine, a N-methyl ethanolamine, N,N-dimethyl-ethanolamine, and the like. The preferred R groups are



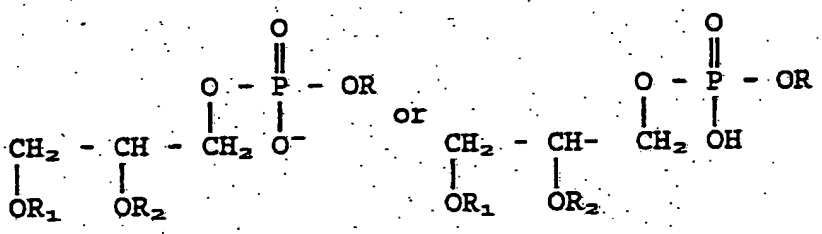
, or



wherein R_8 and R_9 are independently hydrogen or lower alkyl, and preferably hydrogen. The most preferred are $CH_2CH_2NH_2$ and $-CH_2CH_2\overset{\oplus}{N}(CH_3)_3$.

The prefix "sn-" as employed herein is used to denote the carbons of the glycerol backbone of the fatty acid according to the stereospecific numbering system established for lipid nomenclature. In other words, sn-1 denotes the carbon at the first position, sn-2 denotes the carbon at the second position, etc.

The phospholipid ester depicted hereinabove can exist in two forms,



The term "basic salts" contemplates the former form, wherein basic salts are as defined herein. Unless specified to the contrary, the drawing of one form also contemplates to the other form.

The carbon atom at the sn-2 position of the phospholipid ester depicted hereinabove contains an asymmetric center. Thus, the phospholipid ester as well as carbon atoms may exist in two stereochemical configurations, the L-stereoisomeric form (the natural

configuration) or the D- form. Both stereoisomeric forms are contemplated by the present invention.

It is to be noted that when R_2 or R_1 is hydrogen, these compounds represent the lysophospholipids of the present invention.

Of course, there may be additional chiral centers present on the R, R_1 and R_2 groups, which also gives rise to various stereoisomeric forms. These various stereoisomeric forms are also contemplated to be within the scope of the invention. Therefore, all of the various configurations around each chiral center present in the phospholipid compounds of the present invention, including the various enantiomers and diastereomers as well as racemic mixtures and mixtures of enantiomers and/or diastereomers of the compounds of the present invention, either singly or in combination, are contemplated by the present invention.

It is preferred that when R_2 or R_1 is other than hydrogen, R_2 and R_3 are not branched, but are straight chained.

It is preferred that the hydrocarbyl group of the heteroatom fatty acid, i.e., the aliphatic portion of the heteroatom fatty acid, is saturated. When R_2 or R_1 is other than hydrogen, they may be heteroatom fatty acid acyl group or an acyl group of a fatty acid, as defined herein.

When R_1 and R_2 are a fatty acid acyl group, the fatty acid acyl group may have, in one embodiment, 4-8 carbon atoms, or in another embodiment 13-14 carbon atoms. It is also preferred that the alkyl fatty acid acyl group be a hydrocarbyl fatty acid acyl group. Additionally, in an embodiment of the present invention,

the fatty acid acyl group has a carboxy substituent at the omega carbon. It is preferred also that R_1 and R_2 be straight chained.

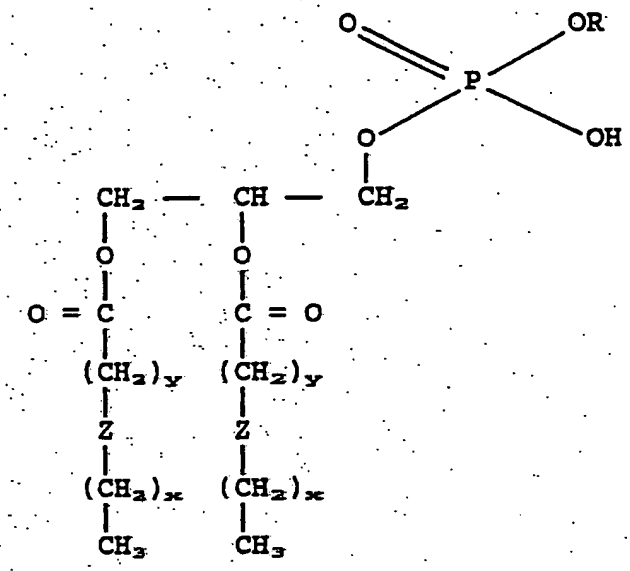
When R_2 or R_1 is a heteroatom fatty acid acyl group, then this group has the characteristics as defined herein. It is preferred that the heteroatom fatty acid acyl group contain no carbon-carbon multiple bonds. In one embodiment, it is preferred that R_2 and R_1 consist of a hydrocarbyl moiety attached to the acyl. It is preferred that the hydrocarbyl group is saturated. It is further preferred that the hydrocarbyl group be a straight chain. The heteroatom hydrocarbyl group may contain more than one oxygen atom, sulfur atom or combination thereof in the principal chain, although oxygen is the preferred heteroatom. It is more preferred, however, that the principal chain contain only one oxygen or sulfur. Furthermore, it is preferred that the heteroatom not be α to the acyl group or be on the omega position of the chain. It is most preferred that there may be only one heteroatom in the principal chain and that the heteroatom is oxygen.

Another preferred value of R_1 and R_2, when defined as a heteroatom fatty acid acyl group, is the 1-substituted alkyl fatty acid acyl groups containing 3-25, carbon atoms and the substituents are hydroxy, halo, lower alkoxy, mercapto or alkylthio. In a more preferred embodiment, the substituent is hydroxy or halo, preferably bromo or chloro. In the most preferred embodiment, the hydrocarbyl chain contains 13-15 carbon atoms and most preferably 13-14 carbon atoms and is 1-substituted hydroxy or halo.

Of course, various combinations and permutations are possible, as described below, in which R_1 and R_2 are independently heteroatom alkyl group, 1-substituted alkyl group or heteroatom 1-substituted alkyl group. These various combinations and permutations are contemplated to be within the scope of the present invention.

Preferred embodiments of the present invention are directed to compounds wherein R is as defined hereinabove, one of R_1 and R_2 is hydrogen, alkyl fatty acid acyl group having 4-26 carbon atoms, or alkyl heteroatom fatty acid acyl group having 13-14 carbon atoms, and the other is independently a heteroatom hydrocarbyl fatty acid group containing 13-14 carbon atoms containing at least one oxygen or sulfur.

In a further embodiment, the present invention contemplates a phospholipid drug of the formula:



VIII

wherein R is as defined hereinabove;

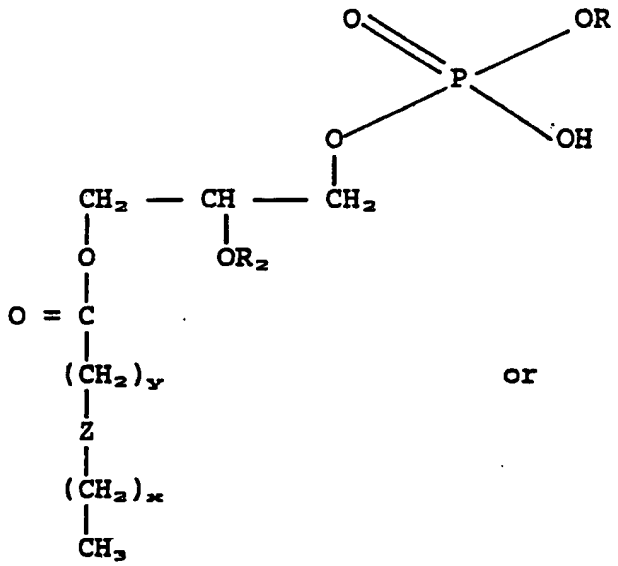
Z is oxygen or sulfur;

each x is independently 0 to 13;

each y is independently 1 to 13; and

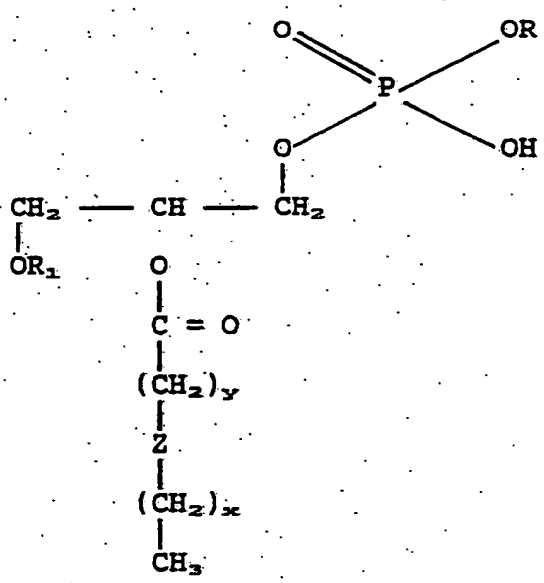
x + y = 11-15 and most preferably 11.

The present invention also contemplates a phospholipid drug of the formulae:



or

IX



IXA

wherein R is as defined hereinabove;

R_2 is hydrogen or alkyl fatty acid acyl group having 4-26 carbon atoms, and more preferably 4-8 or 14 carbon atoms,

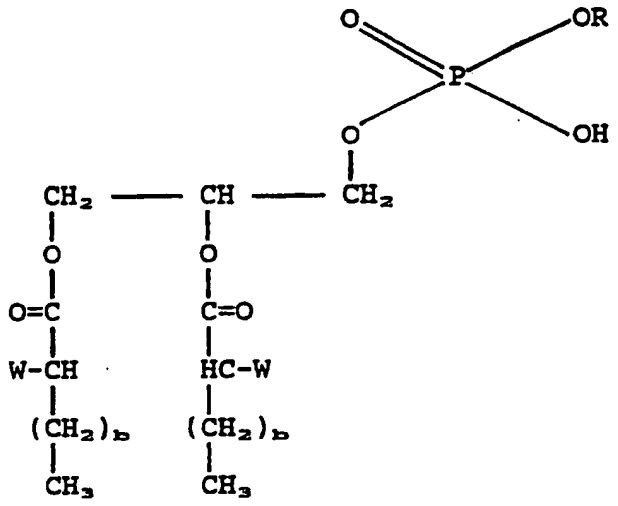
Z is oxygen or sulfur;

each x is independently 0-13;

each y is independently 1-13; and

x + y = 11-13, and most preferably 11.

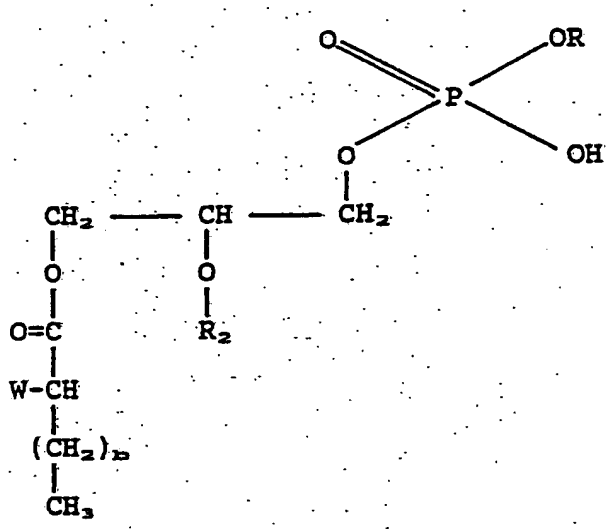
In a further embodiment, the present invention relates to a phospholipid drug of the formula:



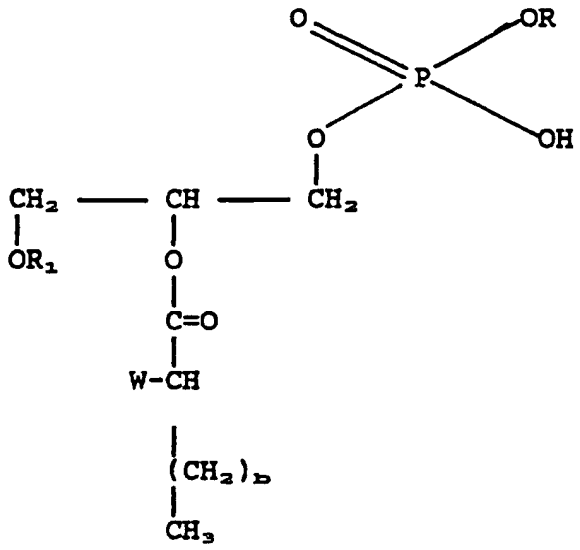
X

wherein W is halo, hydroxy, alkoxy, mercapto or alkylthio; and R is as defined hereinabove and b is 11-13 and most preferably 11.

Another preferred embodiment of the present invention relates to a phospholipid drug of the formulae:



XI or



XII

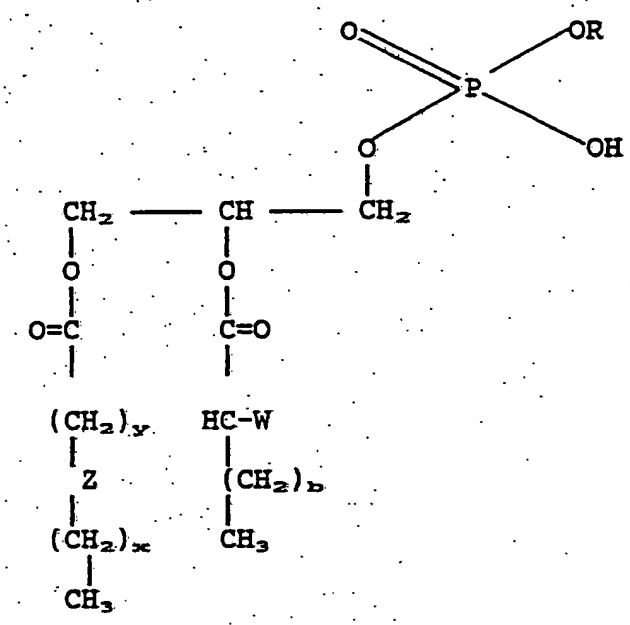
wherein R is as defined hereinabove;

R_1 and R_2 are independently hydrogen or alkyl fatty acid acyl group having 4-26 carbon atoms and more preferably 4-8 or 14 carbon atoms,

b is 11-13, and most preferably 11

and W is selected from halo, alkoxy, mercapto or alkylthio.

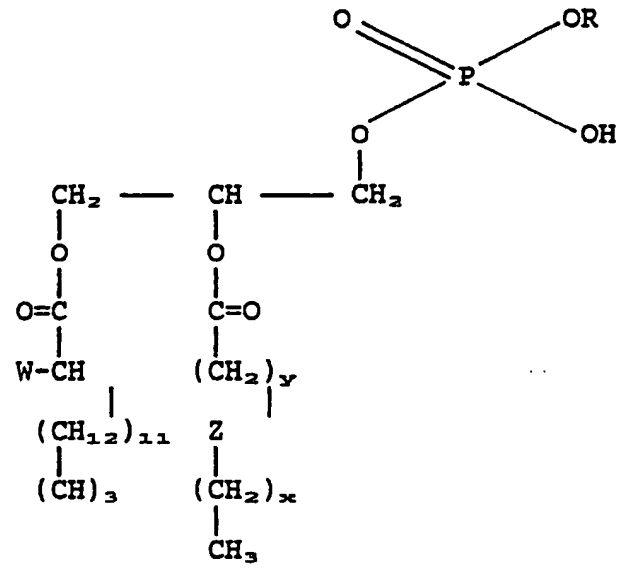
In a still further embodiment, the present invention relates to a phospholipid drug of the formula:



XIII

wherein R, Z, W, x, y and b are as defined hereinabove.

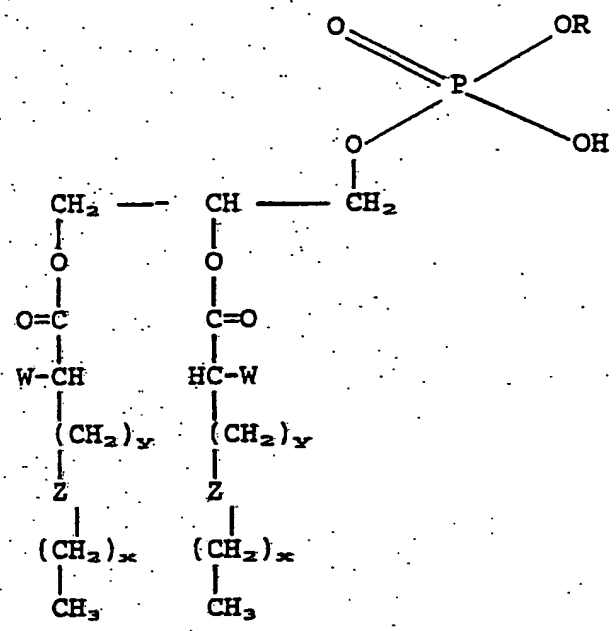
Another embodiment of the present invention contemplates a phospholipid drug of the formula:



XIV

wherein R, Z, W, x and y are as defined hereinabove.

In a further embodiment, the present invention contemplates a phospholipid drug of the formula:



XV

wherein R, Z, W, x, y are each as defined hereinabove. The compounds described in Formulae VIII to IX also contemplate the basic salts, as defined herein.

In the various embodiments described herein, it is preferred that R is

H;

$CH_2\ CH_2 - \overset{\oplus}{N}-(CH_3)_3$;

$-CH_2\ CH_2 - NH_2$,

$-CH_2\ \underset{\underset{OH}{|}}{CH} - CH_2 - OH$ or

$-CH_2\ \underset{\underset{NH_2}{|}}{CH} - COOH$.

Preferably, R is

$$-CH_2-CH_2-\overset{\oplus}{N}(CH_3)_3 \quad \text{or}$$

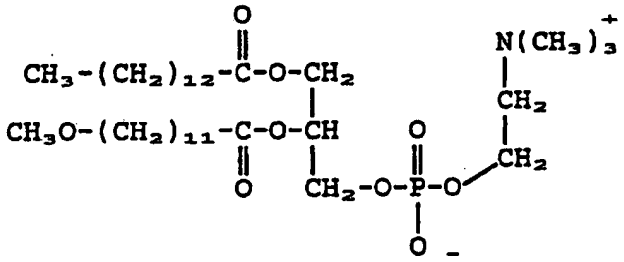
$$CH_2 - CH_2 - NH_2.$$

In all the embodiments contemplated in Formula VIII-XV hereinabove, it is most preferred that Z is O or S, x = 0, y = 10 or 11 and W is hydroxy.

In the embodiments described hereinabove, all of the various combinations and permutations of the various variables, R, R_1, R_2, W, Z, x, y, b, etc., wherever possible, is contemplated by the inventors. Furthermore, the present invention encompasses embodiments (compounds, methods, compositions, etc.) which contain one or more elements of each of the Markush groupings in R, R_1, R_2, W, Z, x, y, b, etc. and the various permutations and combinations thereof.

In still another embodiment, the present invention contemplates the compound 1-myristoyl-2-(12-methoxydodecanoyl)-sn-3-phosphalidylcholine (AC1) represented by the formula:

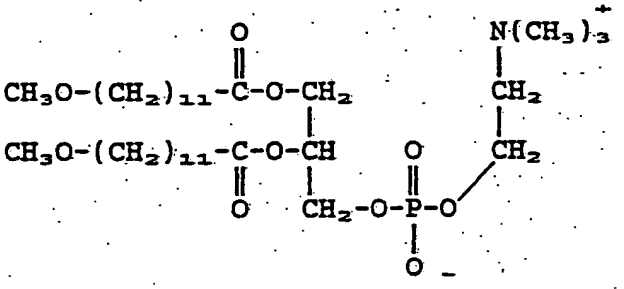
AC1



As clearly seen, AC1 contains the fatty acid 12MO bonded to the sn-2 position of the glycerol backbone, while a myristoyl group is bonded at the sn-1 position. The present invention contemplates both the L- and D-stereoisomers.

In another embodiment, the present invention contemplates the compound 1,2-(di-12-methoxydodecanoyl)-sn-3-phosphatidylcholine (L-AC2) represented by the formula:

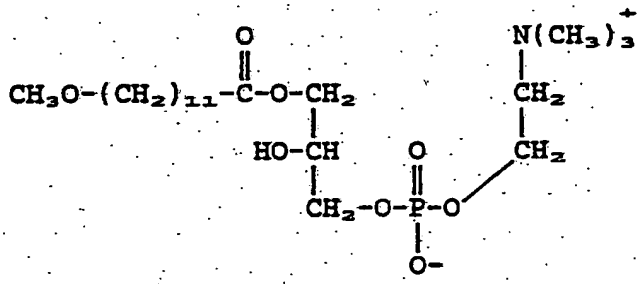
AC2



Compound AC2 contains the fatty acid 12MO bonded to both the sn-1 and sn-2 positions of the glycero backbone. Furthermore, the configuration at the sn-2 carbon of the glycero backbone is in the L configuration.

The present invention also contemplates the D-AC2 molecule, wherein the configuration at the sn-2 carbon of the glycerol backbone is in the D configuration.

In still another embodiment, the present invention contemplates the compound: 1-(12-methoxydodecanoyl)-sn-3-glycerophosphatidylcholine represented by the formula:



hereinafter referred to as the "lysolipid analogs". The lysolipid analog contains the fatty acid 12MO bonded to the sn-1 position and hydrogen bonded at the sn-2 position of the glycerol backbone. Alternatively, the lysolipid analogs may contain the heteroatom fatty acid, (e.g., 12MO) bonded to the sn-2 position and the hydrogen bonded to the sn-1 position. Again, both the D- and L-stereoisomers contemplated by the present invention.

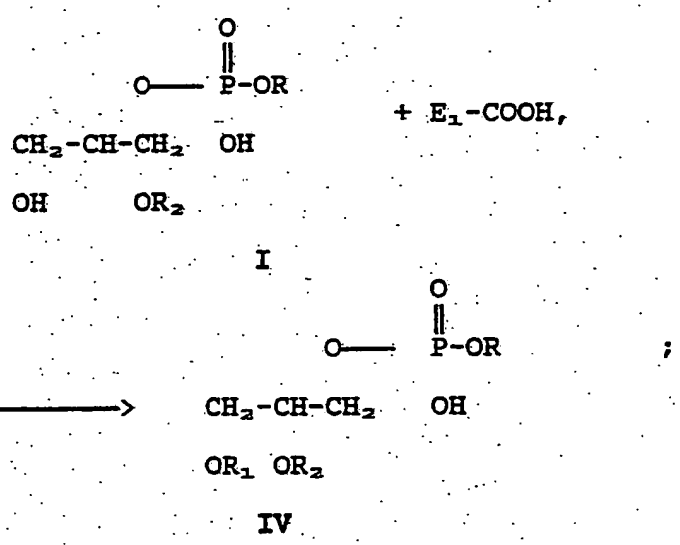
Compounds of the present invention can be prepared in accordance with art- recognized techniques. Exemplary procedures are described below.

The carboxy group on the heteroatom fatty acid is activated by standard methods for acylation and is reacted with the hydroxy group on the glycerol backbone under esterification conditions, known to one skilled in the art. The reaction may be run in inert solvents that will dissolve both reagents or it may be run in a biphasic solvent. Examples include DMSO, crown ethers and the like. The reaction is run at temperatures facilitating acylation. These temperatures may range from room temperature to the reflux temperature of the solvent, although it is preferred that the reaction is run at about room temperature or slightly above. Furthermore, the reaction may be run under reduced pressure, such as under vacuum.

Alternatively, the reaction may be run by first converting the acid to an acylating derivative, such as the acid halide (e.g., acid chloride, acid bromide) or anhydride, under reaction conditions known to one skilled in the art. The acylated derivative is then reacted with the hydroxy group on the glycerol

backbone of the glycerol phospholipid under esterification conditions as described hereinabove. In other words, the reaction may be run in an inert solvent that will dissolve both regents or it may be run in a two-phase solvent system. The reaction is run at temperatures facilitating acylation. These temperatures may range from room temperature to the reflux temperature of the solvent, although it is preferred that the reaction is run at about room temperature or slightly above. Further, the reaction may be run under reduced pressure.

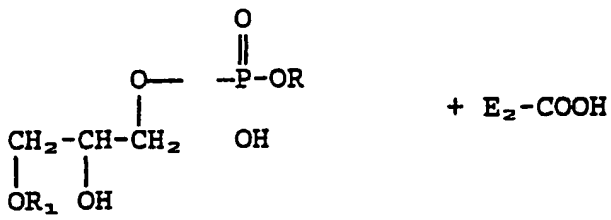
The reactions described hereinabove can be schematically represented as follows:



I

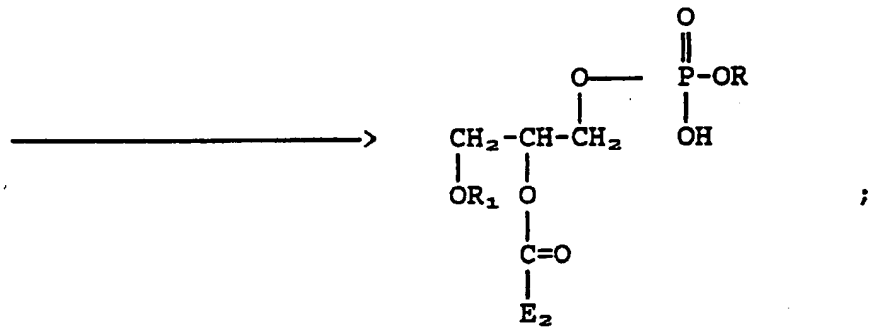
IV

wherein E_1C is R_1 and is a heteroatom fatty acid acyl group; E_1 COOH is a heteroatom fatty acid



+ E_2-COOH

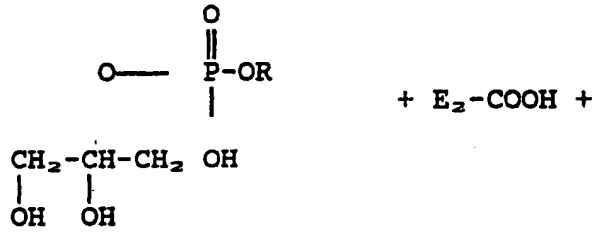
II



;

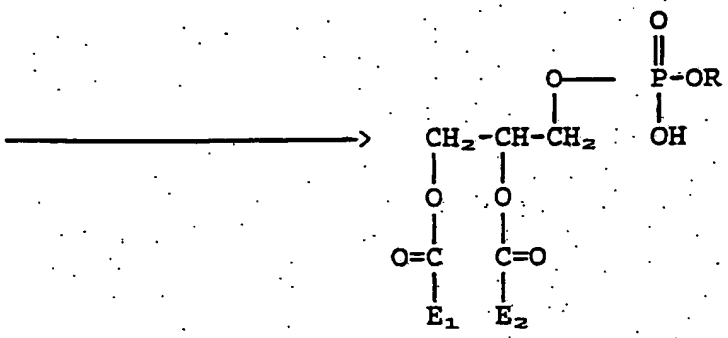
V

wherein E_2C(=O) is R_2 and is a heteroatom fatty acid acyl group; and E_2COOH is a heteroatom fatty acid



+ E_2-COOH + E_1COOH

III



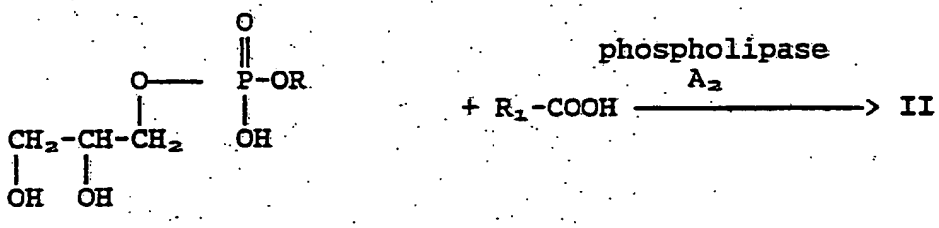
VI

wherein C - E_1 is R_1 and C - E_2 is R_2, and E_1 COOH and E_2 COOH are defined hereinabove.

In the above schemes, R_1, R_2 and R are as defined hereinabove and E_1 COOH and E_2 COOH are the heteroatom fatty acids, as defined herein.

Compounds of Formula III are either available commercially or can be prepared by art recognized methods.

Compounds of Formula II wherein R_1 is other than hydrogen can be prepared from compounds of Formula III as follows:



III

Acylation of III with an excess of acylating derivative of R_1-COOH (e.g., the acid halide, anhydride or acid) under esterification conditions will produce the diacylated compounds. The esterification conditions are similar to those described hereinabove. Hydrolysis of the acylated compound with phospholipase A_2 will produce the compound of Formula II.

Similarly, compounds of Formula I wherein R_2 is other than hydrogen can be prepared from compounds of Formula III as follows:

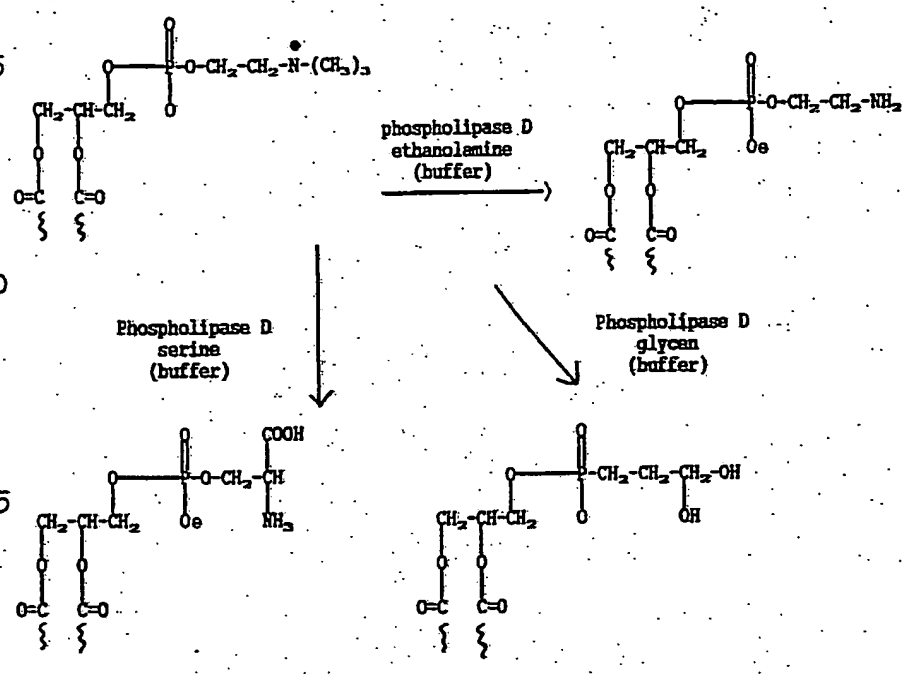
$$\text{III} + E_2\text{-COOH} \xrightarrow{\text{phospholipase } A_1} \text{I}$$

Acylating III with an excess of an acylating derivative of E_2-COOH (e.g., the acid halide, anhydride or acid) under esterification conditions as described hereinabove will produce the diacylated compounds. Hydrolysis of the diacylated compounds with phospholipase A_1 will produce the compound of Formula I.

In both cases described hereinabove, the acid halide can be prepared from the corresponding acid with thionyl chloride or bromide. Similarly, the anhydride can be prepared from the corresponding acid by reacting the acid with a dehydrating agent, such as P_2O_5 or dicyclohexyl-carbodiimide. Alternatively, the anhydride can be prepared by reacting the acid halide with the corresponding salt of the acid.

Furthermore, diacylated analogs containing either a glycerol (PG), serine (PS) or ethanolamine (PE) headgroup can be synthesized by transphosphatidylation

using phospholipase D treatment of diacylated phosphatidyl choline analogs, as described hereinbelow.

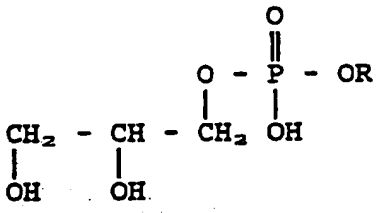


wherein represents the remainder of the fatty acid chain. The transphosphatidylation with phospholipase D is effective with both the L- and D- stereoisomers of the glycerophospholipid. Cleavage with phospholipase A_1 or phospholipase A_2 of the L-isomer will produce the 1-hydroxy or 2-hydroxy analog, respectively.

The acylating derivative of the heteroatom fatty acid can be prepared in accordance with art-recognized procedures. For example, the acid chloride can be prepared by reacting the fatty acid with thionyl chloride. The anhydride can be prepared by reacting the fatty acid containing a free carboxy group with a dehydrating agent, such as P_2O_5 or

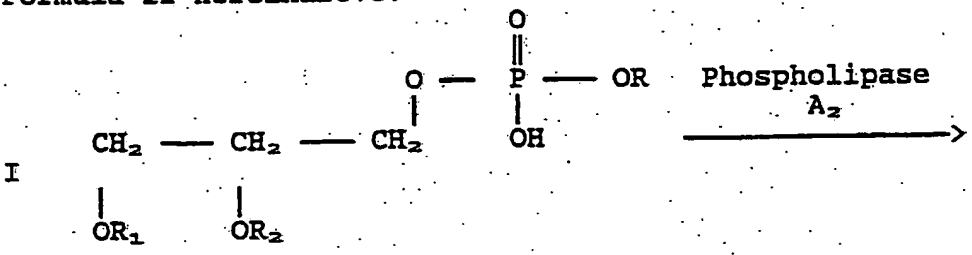
dicyclohexylcarbodiimide, acetic anhydride, trifluoroacetic anhydride, methoxyacetylene and the like. Alternately, the anhydride can be prepared by treating the acid halide (such as acid chloride) of the fatty acid with the acid salt of the drug.

Alternatively, and especially in the case of the phospholipid drugs of myristic acid derivatives, the phospholipid drug can be prepared using immobilized artificial membranes (IAM), as described in Markovich, et al. in Anal Chem., 1991, 63, 1851-1860, the contents of which are incorporated herein by reference. The procedure will be described in more detail hereinbelow. Generally, the glycerol phospholipid of the formula:



(hereinafter referred to as GP) wherein R is as defined hereinabove (1 mMol) is solubilized in MeOH (0.5-2.0ml) and is adsorbed onto an IAM packing material, prepared as described hereinbelow by dropwise addition of the methanolic-PC solution. The MeOH was allowed to evaporate after the IAM surface was completely loaded with GP. The IAM/GP solid material was dried overnight in a vacuum at 45°C. After drying, the IAM/GP powdered was suspended in dry chloroform containing the dried acylating derivative of the drug (acid halide, anhydride, and the like) and dried equivalent of a catalyst, such as dimethylaminopyridine and the like.

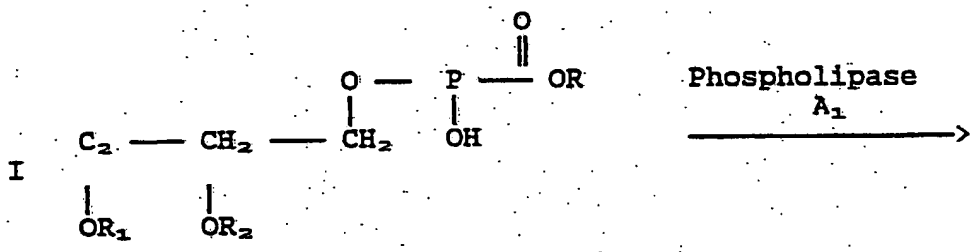
The monoacylated phospholipid compounds of the present invention (lyso form) can also be prepared from the diacylated phospholipids by using the appropriate phospholipase. For example, phospholipase A_2 selectively hydrolyzes the ester at the sn_2 position of the glycerol backbone to form a compound having the Formula II hereinabove:



I

In the above scheme, R, R_1 and R_2 are as defined hereinabove, except that R_1 and R_2 are not hydrogen.

Similarly, the other lyso form having Formula I can be prepared from the diacylated phospholipid by using phospholipase A_1, which selectively hydrolyzes the ester at the sn_1 position of the glycerol backbone:



I

A

Finally, the compound of Formula IV can be prepared by hydrolysis of I with phospholipase A_2, hydrolysis of II with phospholipase A_1 or hydrolysis of A with phospholipase A_1 and phospholipase A_2 in either

order. It is to be noted that in the above schemes, R, R_1 and R_2 are as defined hereinabove, except that R_1 and R_2 are not hydrogen.

In the acylation reactions described above, E_1 COOH or E_2 COOH may be unsubstituted or substituted. If substituted, it is preferred that the substitution is on the α carbon (the carbon atom adjacent to the carboxy group). Further, the preferred substituents are hydroxy, lower alkoxy, mercapto or alkyl thio.

These compounds can be prepared from art recognized techniques. For example, the α-hydroxy compound can be prepared from the corresponding α-halo carboxylic acid by reacting the latter with base (OH-) under substitution reaction conditions. Furthermore, the mercapto compound can be prepared from the corresponding α-halo carboxylic acid by reacting the latter compound with HS^- under substitution reaction conditions, while the α-alkylthio carboxylic acid can be prepared from the corresponding α-halo carboxylic acid by reacting the latter with lower alkylthiolate under substitution reaction conditions. The α-lower alkoxy derivative can be prepared by reacting the α-halo carboxylic acid with lower alkoxide under Williamson reaction conditions.

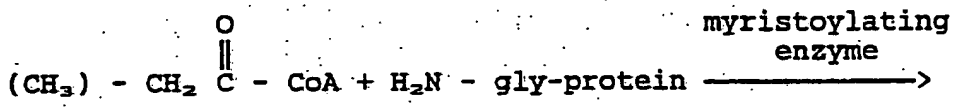
The α-halo carboxylic acid can be prepared by reacting E_1 COOH (or E_2 COOH) with phosphorous and halogen (preferably Cl_2 or Br_2) or phosphorus trihalide under Hell-Volhard-Zelinsky reaction condition as described hereinbelow on Pages 53-54. Alternatively, the α-halo carboxylic acid can be prepared from the malonic acid ester synthesis described hereinbelow.

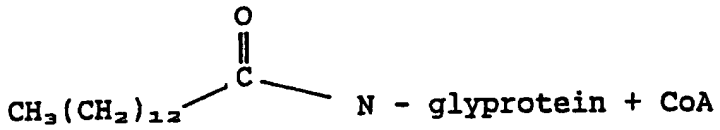
It is to be understood that in some of the reactions described hereinabove, it may be necessary to employ protecting groups on reactive functional groups, such as hydroxy, that may be present. The protecting groups to be employed are obvious to one skilled in the art. Examples of various protecting groups can be found in "Protective Groups in Organic Synthesis" by T.W. Green, John Wiley and Son, 1981, which is incorporated herein by reference.

In the reactions described hereinabove, the various products can be separated and purified by art recognized techniques known to one skilled in the art, such as flash chromatography or HPLC.

The phospholipid compounds resulting from the above reactions are used to treat diseases caused by retroviruses, such as AIDS and ARCS in animals, especially mammals, by administering to said animal an effective amount of the compound to treat said diseased state. The present invention is directed to those compounds as well as the use of the compounds in treating diseases caused by retroviruses. Without wishing to be bound, it is believed that the compounds of the present invention interfere with protein myristoylation, a reaction which is necessary for HIV infections.

The HIV-1 genome encodes for two myristoylated proteins: p^{17gag} and p^{27nef}. In situ myristoylation of these proteins is critical for the establishment and maintenance of HIV infection. The myristoylation reaction can be represented as follows:





N-myristoylatransference (NMT) is the enzyme that cotranslationally transfers the myristoyl group to endogenous cellular and viral proteins. It is believed that the compounds of the present invention exhibit inhibitory activity against viruses that produce myristoylated proteins.

Interference with protein myristoylation has been a drug target site for inhibiting HIV replication. It has been reported that heteroatom analogs of myristic acid containing oxygen or sulfur substituted for alkyl methylene groups exhibit activity against HIV replication in infected cells. European Patent Application 415,902 alleges that oxy and thio substituted fatty acid analog substrates of myristoylating enzymes in which a methylene group at carbon position 4 to 13 is replaced by an oxygen or sulfur can be used to treat retroviral infections. It has also been reported that metabolic activation of 2-substituted derivatives of myristic acid inhibits myristoyl CoA: Protein N-myristoyltransferase. See Paige, et al., Biochemistry 1990, 29, 10566-10573.

However, the present inventors have discovered that the efficacy of these compounds have been significantly enhanced by acylating these molecules to the glycerol backbone of a phospholipid in accordance with the present invention, thus generating new phospholipid drugs. More particularly, the fatty acid analogs of myristic acid are acylated to one or both of

the hydroxy groups of the glycerophospholipid, i.e. the non-polar end. The acylation at the non-polar end of the phospholipid significantly influences the ability to inhibit HIV replication in macrophages and T cells and also alters the toxicity of the fatty acid analogs. Additionally, these acylated phospholipids may be sensitive to phospholipases A1 and A2, thereby providing a specific cleavage mechanism for the acyl group(s) containing the biologically active fatty acids, once the product is transported into the cell.

The present compounds can be formulated with suitable pharmaceutically acceptable carriers into unit dosage form and can be administered orally, transdermally parenterally or rectally. The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers and the like. Such compositions and preparations should contain at least 1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains a

pharmaceutically effective amount which can be determined by the physician. For example, the oral dosage unit form may contain between about 0.5 and 1000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such a peppermint, oil of wintergreen or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

The active compound may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary

conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene, glycol, and liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in unit dosage form for ease of administration and uniformity of dosage unit. Dosage form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined

quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. The physician can determine the amount of drug to be utilized. A unit dosage form can, for example, contain the principal active compound in amounts ranging from about 0.5 to about 1000 mg. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The general descriptions above are specifically illustrated hereinbelow with representative examples, AC-1, AC-2 and the lysoanalogs thereof. The following application of the generalizations hereinabove are provided solely for illustrative purposes. The invention is not to be limited in any way by the exemplification hereinbelow. The AC1 and AC2 and the lyso compound are the most preferred embodiments of the present invention and can be prepared by art recognized

synthetic procedures. Exemplary schemes are as outlined below.

Heteroatom-fatty acids

The general synthetic scheme for synthesizing hetero-atom-fatty-acids is outlined in EPA 0,415,902, which is incorporated herein by reference.

Synthetic scheme:

$$CH_3(CH_2)_xZ^- + L\text{-}(CH_2) - (CH_2)_{y-1} - COOH \xrightarrow{CH_3(CH_2)_xZH}$$

XVI XVII

$$CH_3(CH_2)_xZ - (CH_2)_y - COOH$$

wherein x, y and Z are as defined hereinabove and L is a leaving group, such as halo, OTS, OMS and the like. A base having Formula XVI is reacted with a carboxylic acid of Formula XVII under Williamson-like conditions in $CH_3(CH_2)_xZH$. The reaction is run at effective temperatures, which may range from room temperature up to reflux temperatures, although it is preferred that the reaction be carried out under reflux temperatures. The following example illustrates the formation of the heteroatom fatty acids.

The general synthetic scheme for obtaining 12 MO is outlined below. A flame dried 300-ml round bottom flask was cooled before 8.4 g (0.030 mol) of 12-bromododecanoic acid was mixed with 6.5 g (0.120 mol) sodium methoxide in 200 ml of absolute methanol. The yellow solution was refluxed at 85°C for 16-20 hours under a nitrogen atmosphere. After refluxing, the mixture was allowed to cool and the solvent was removed

by rotoevaporation. After removing most of the solvent, approximately 2-4 milliliters of residue remained and was extracted by the addition of ethyl acetate 100 ml, ether 50 ml and H_2O 50 ml. Prior to acidification, the organic layer was clear and the top aqueous layer was yellow. This organic/aqueous mixture was acidified to pH 3 with 1 N HCl causing the organic layer to yellow and the aqueous layer to become clear. The aqueous and organic layers were separated and the aqueous layer was extracted twice with 30-50 ml of ethyl acetate. The organic extracts were pooled and washed once with 50 ml of H_2O. The organic layer was dried using anhydrous Na_2SO_4 and filtered. After removing the solvent by rotoevaporation, the residue was heated (50°C) under vacuum for 5 hours to remove trace organic solvents. TLC analysis using ethyl acetate:hexanes:formic acid 88:9:3, gave rf = 0.25. Typical yields range from about 80-95%.

<u>Hetero-atom-fatty-acid anhydrides</u>

The corresponding anhydride of the heteroatom fatty acid is formed by coupling the heteroatom fatty acid with a dehydrating agent, such as dicyclohexylcarbodiimide, as illustrated by the exemplary procedure hereinbelow.

The anhydride was prepared in a flame dried 50-ml round bottom flask containing 5.4 g (0.024 mol) of 12-methoxydodecanoic acid completely dissolved in 20 ml of dry THF under a nitrogen atmosphere. After adding DCC 2.40 g (0.012 mol) dropwise over 5 minutes, the reaction was complete in under 25 minutes as monitored by the disappearance of the DCC imine vibration band

(centered at 2100 cm^{-1}). Preliminary studies showed if solid DCC was added to the reaction mixture then several side products were found by TLC. Consequently DCC was melted, weighed into a flame dried beaker, and diluted with 5 ml of dry THF. Dicyclohexylurea (DCU) precipitates within the first few minutes of DCC addition. DCU was removed by paper filtration (Whatman #1). The solvent was removed by rotoevaporation and placed under a heated vacuum (50°C) for 12 hours. FTIR and TLC analysis (ethyl acetate:hexanes:formic acid 88:9:3) revealed no DCU or DCC in the final product. Typical yields were 90-95%.

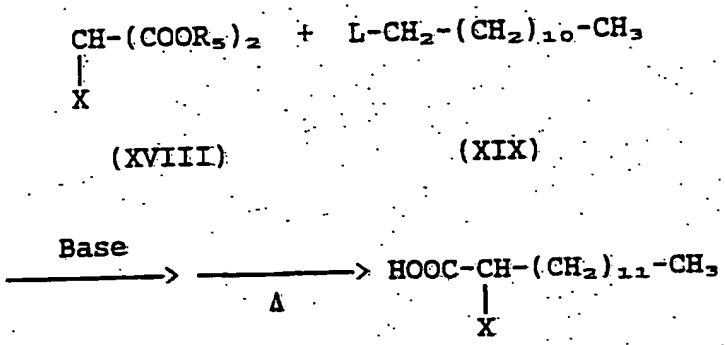
Substituted myristic acid analogs

The synthesis of the substituted myristic acid is also prepared by art recognized techniques. Although the reaction substrate described hereinbelow is myristic acid, the following examples are exemplary and are applicable to fatty acids in general.

The fatty acid can be halogenated as follows:

$$CH_3(CH_2)_{12}COOH \xrightarrow[PX_3]{X_2,P \text{ or}} CH_3(CH_2)_{11}CHCOOH \quad (X)$$

wherein X is halo, e.g., bromo or chloro. The fatty acid is halogenated with phosphorus in the presence of halogen, or with PX_3 under Hell-Volhard-Zelinsky reaction conditions to form the α-halogenated product. Alternatively, the acid may be formed in two steps using a variation of the malonic ester synthesis:



(XVIII) (XIX)

In the above scheme, X is halo, R_5 is lower alkyl, such as methyl, ethyl and the like, and L is a better leaving group than X, such as OTS, OMS and the like. For example, if X is F, then L may be OTS, OMS, Br, I and the like.

As described hereinabove, the halo malonic acid ester (XVIII) is reacted with a strong base to remove the acidic hydrogen on the α-carbon. The resulting anion is then reacted with an alkyl halide (XIX) and forms the coupled diester. The reaction is run in an inert solvent, such as dimethyl formamide, and preferably under anhydrous conditions. The resulting product is then heated at temperatures effective for decarboxylation to form the final product.

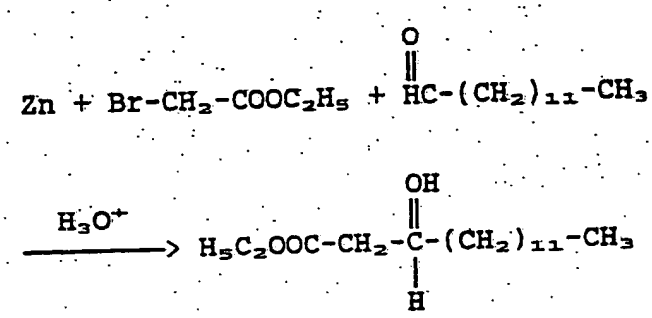
The reaction is further exemplified by the following example.

The general synthetic scheme for synthesizing substituted myristic acid analogs is similar to the synthesis of 2-fluoromyristic acid which can be synthesized as follows: To a suspension of 0.32 g (11.2 mmol) of an 80% oil dispersion of NaH in 8 mL of dry DMF was added dropwise 2 g (11.2 mmol) of diethyl fluoromalonate under argon. The suspension was then stirred for 4.5 h after which time, 2.79 g (11.2 mmol)

of 1-bromododecane was added and the solution was heated at 90°C for 18 h. The yellow suspension was then poured into 10 mL of water and extracted with ether (2 x 15 mL). The combined ether layers were washed, dried and evaporated. A yellow oil resulted, which was used for the next reaction without further purification. A mixture of 3.8 g of the crude diethyldodecylfluoromalonate (8), 30 mL of 6 N HCl, and 50 mL of dioxane was refluxed for 72 h. After cooling, the yellow solution was dissolved in 100 mL of petroleum ether (bp 40-60°C). The organic layer was separated and washed with water (3 x 50 mL) and 10% KOH (2 x 250 mL). The combined aqueous layers were acidified to pH 1.0 with concentrated HCl and extracted with ether (3 x 100 mL). The ether layer was dried, filtered and evaporated to dryness to yield a green solid. The solid was decolorized with activated carbon and recrystallized from petroleum ether (bp 40-60°C) to give 1.501 g (54% overall yield) of 2-fluoromyristic acid (2) as white needles.

2-bromomyristic acid was purchased from Aldrich and 2-hydroxymyristic acid was purchased from Fluka.

A beta hydroxy acid can be formed by reacting a ß-halo ester with an aldehyde in the presence of zinc under Reformatsky reaction conditions in an inert solvent, such as toluene or DMF.



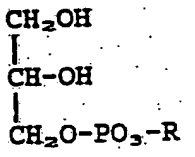
The resulting product is then hydrolyzed to form the corresponding acid.

The ß-hydroxy compound can then be converted to the corresponding halide by art recognized techniques, such as reaction with thionyl bromide or chloride, phosphorous trihalide, (SO_2Cl_2, SO_2Br_2, PCl_3, PI_3) and the like.

The corresponding anhydrides of the substituted fatty acid analog can be prepared by coupling the substituted fatty acid analog prepared hereinabove with a dehydrating agent, such as decyclohexylcarbodiimide, as illustrated by the exemplary procedures hereinabove.

Phospholipids

The phospholipids are prepared by art recognized techniques by reacting an acylating derivative of the fatty acid, such as the fatty acid anhydride, with the glycerol phosphate of the formula:



wherein R is as defined hereinabove, under esterification conditions. This reaction is illustrated

hereinbelow. Although the reaction is illustrated using the heteroatom fatty acid to form the heteroatom fatty acid phospholipid, the reactions described hereinbelow are applicable using the substituted fatty acid analogs to form the phospholipid containing the substituted fatty acid analogs.

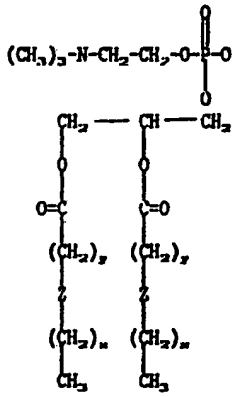
The chemical reaction for the solid phase adsorption synthesis of L-AC2 is given in Rxn. 1. The synthetic route for obtaining the anhydride used in Rxn. 1 is given above.

Rxn.1

Solid Phase Synthesis

Glycerophosphocholine (GPC)

1AH.PC/DMAP.CHCl$_3$



Diacylated phosphatidylcholine analogs

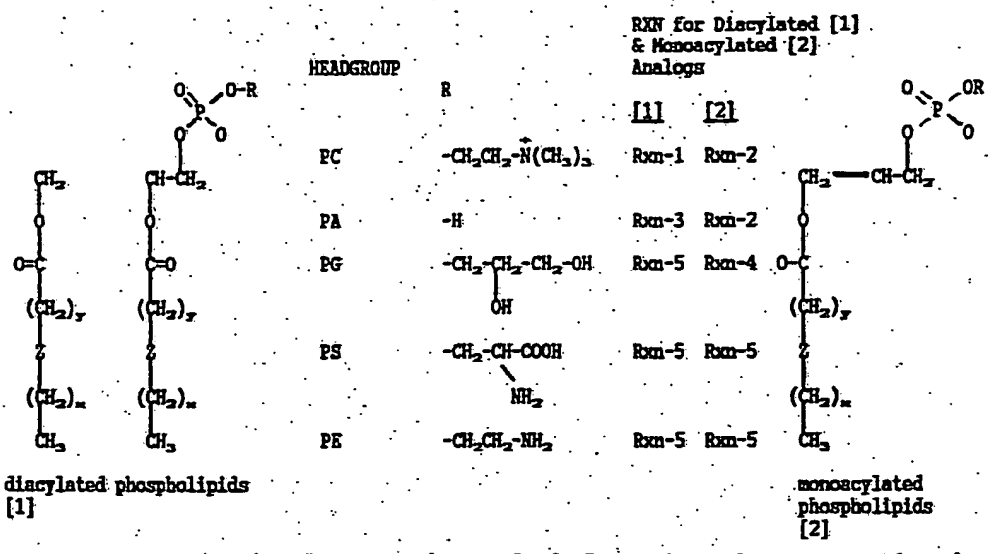
X = 0-11
Y = 0-11
x+y = 11
Z = oxygen or sulfur

Phospholipids are not difficult to synthesize, but the present novel-synthetic-method overcomes several experimental inconveniences associated with phospholipid synthesis. During the synthesis of diacylated phospholipids, an experimental inconvenience involves the insolubility of glycerophosphocholine (GPC) in common organic solvents. Although GPC is soluble in dimethylsulfoxide (DMSO), the use of DMSO requires vacuum distillation and in addition, DMSO makes the purification of phospholipids more difficult. The inventors have developed a novel method for synthesizing molecules when all reactants are not soluble in the same organic solvent. The method involves using a chromatographic surface to promote the interaction between one insoluble reactant and one soluble reactant. In other words, the insoluble-reactant is initially adsorbed to the chromatographic surface, and the soluble-reactant partitions into the chromatographic interface during the reaction. Partitioning of the soluble-reactant between the chromatographic surface and the reaction solvent permits the insoluble molecule, adsorbed at the interfacial region, to react with the soluble reactant. Although other chromatographic surfaces may be useful, IAM.PC chromatographic surfaces were utilized.

The synthesis of AC2 demonstrates the solid-phase-synthetic procedure shown in Rxn. 1. GPC (250 mg, 1 mMol) solubilized in MeOH (0.5 ml), was adsorbed to IAM.PC (200 mg) by dropwise addition of the methanolic-GPC solution; the MeOH was allowed to evaporate after the IAM.PC surface was completely loaded with GPC. The IAM.PC/GPC solid material was dried overnight in a

vacuum oven at 45°C. After drying, the IAM.PC/GPC powder was then suspended in dry $CHCl_3$ containing 1 equivalent of the appropriate anhydride per GPC alcohol, and 1 equivalent of catalyst (i.e., dimethylaminopyridine denoted as DMAP). Both the anhydride and DMAP were dried by vacuum at 45°C. After 6 hours, TLC confirmed the reaction was complete, and the phospholipid product was purified by acetone precipitation and/or silica chromatography. Normally the synthesis would have required 4-5 days per phospholipid and 2 equivalents of anhydride, but this method requires approximately 1-2 days and 1 equivalent of anhydride.

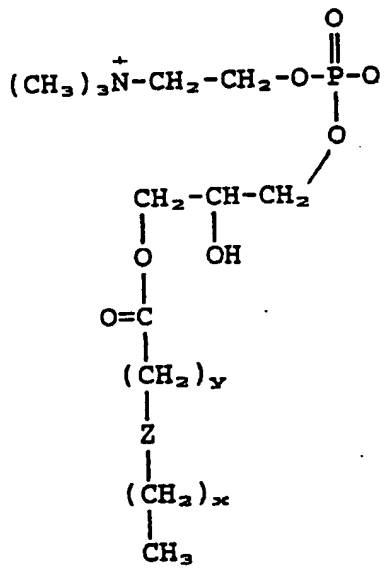
General structures of mono- and diacylated phospholipids whereby all alkyl chains are biologically active fatty acids are shown below. For both the mono- and diacylated phospholipids: x = 0-11; y = 0-11, and x + y = 11 for any given analog. "Z" denotes the hetero-atom and will be either oxygen or sulfur. The chemical reactions for the synthesis of each compound is also given below.



Chemical reactions 2-6 for the above synthetic routes are illustrated below. Briefly, diacylated analogs were prepared by a solid-phase adsorption method (see Rxn. 1 at Pages 54-55 of the application), monoacylated analogs can be prepared from phospholipase A2 treatment of the diacylated compounds, and diacylglycerol analog can be prepared from a separate reaction scheme (i.e., reaction 6). Reaction 6 involves first protecting glycidol epoxide using diphenylsilyl-(t)-butylchloride to form glycidol-tert-butyldiphenylsilyl ether. The epoxide ring is then opened with base, and the protected glycerol is diacylated with 12-methoxydodecanoyl anhydride. The final step in reaction 6 involves deprotecting the diacylglycerol sn-3 alcohol using n-butylammonium fluoride.

Diacylated phosphatidylcholine analogs containing oxygen or sulfur substituted for methylenes can be hydrolyzed with phospholipase A2 to obtain the corresponding lysolecithin analogs.

Rxn.2. [1] + PLA_2 ⟶

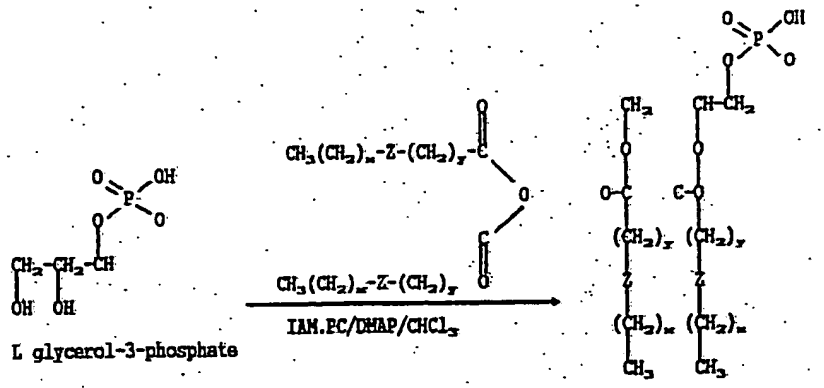


[2]

Monoacylated Phosphatidylcholine

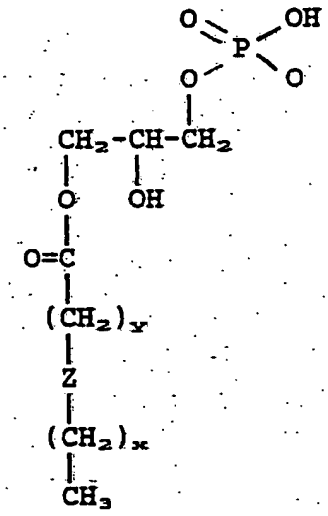
Diacylated phosphatidic acid analogs can be synthesized by reacting L-glycerol-3-phosphate with the appropriate anhydride on the surface of an immobilized artificial membrane particle. This reaction was described in detail in the discussion of reaction 1 using glycerophosphatidylcholine as starting material (see Reaction 3).

Rxn.3. Solid Phase [3] L glycerol-3-phosphate



Monoacylated analogs of phosphatidic acid can be obtained by phospholipase A2 cleavage of the diacylated analogs denoted by [3] in reaction 3. This reaction is described hereinbelow:

Rxn.4 [3] $\xrightarrow{PLA_2}$

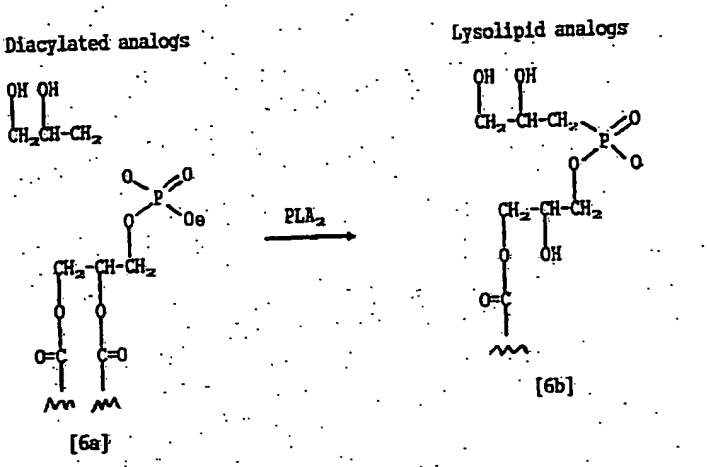


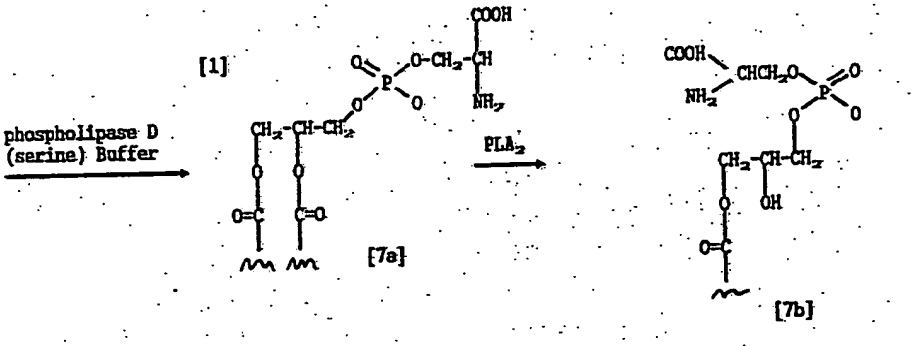
[4]

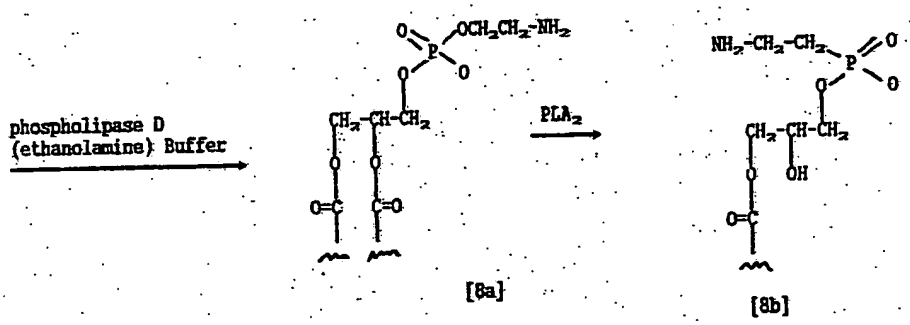
Monoacylated phosphatidic acid

Diacylated analogs containing either a glycerol (PG), serine (PS), or ethanolamine (PE) headgroup can be synthesized by transphosphatidylation using phospholipase D treatment of diacylated phosphatidylcholine analogs denoted as [1] in rxn. 5. The lysolipid analogs with these headgroups can then be obtained by further reaction with phospholipase A2 cleavage.

REACTION 5

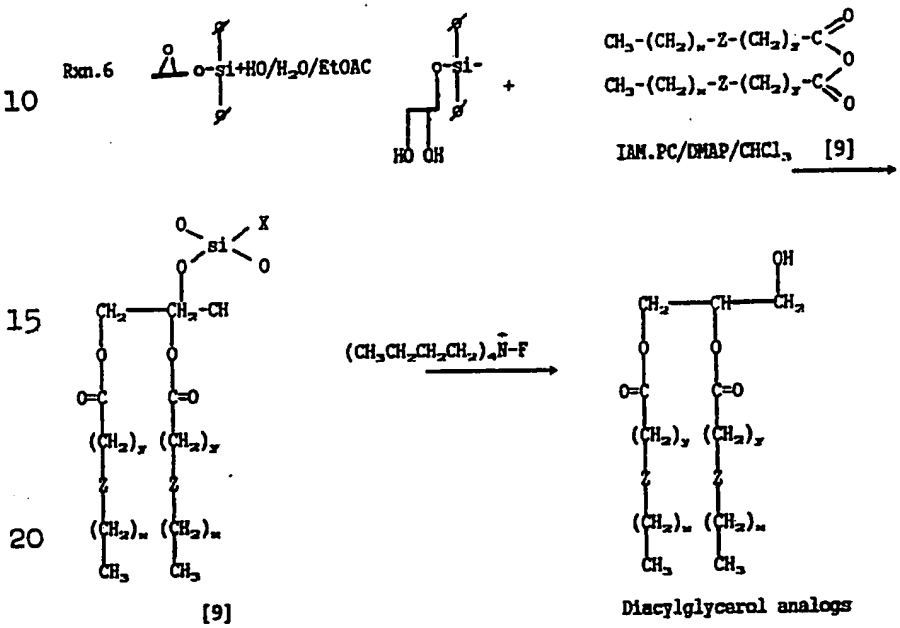






(~) denotes the rest of the fatty acid chain.

Diacylglycerol analogs containing two identical hetero-atom-fatty acids can be synthesized from (t)butyldi-phenylsilyl-O-glycidol. This reaction is performed by solid phase adsorption method employed in reaction 1 using glycerophosphatidylcholine as substrate, as shown below.



EXAMPLES

GENERAL SYNTHESIS

Lecithins are 'diacylated' phospholipids and scheme 1 and scheme 2 shows the general synthetic pathway used to obtain anti-HIV lecithins containing the phosphatidyl-ethanolamine headgroup. Scheme 1 shows the general synthetic scheme used to prepare a lipid containing two 12-methoxy-dodecanoyl groups and a phosphatidylethanolamine headgroup; this lipid is denoted as di-12MOGPE. Briefly, L-α-GPE was reacted with Fmoc-NHS to form GPE-Fmoc in a mixed solvent system; this reaction protected the 1° amine of GPE. GPE-Fmoc was then acylated with 12-MO-anhydride in dry chloroform to form the diacylated product (di-12MOGPE-Fmoc). Di-12MOGPE was then obtained by removing Fmoc with piperidine. Scheme 2 shows the general synthetic scheme used to prepare the anti-HIV lecithin containing one 12-methoxydodecanoyl group and one saturated fatty acid. Briefly, monomyristoylphopha-tidylethanolamine (MMPE) was reacted with Fmoc-NHS to form MMPE-Fmoc; this reaction protected the 1° amine of MMPE. MMPE-Fmoc was then acylated with 12-MO-anhydride in dry tetrahydrofuran to form the diacylated product 1-M-2-12MOGPE-Fmoc. 1-M-2-12 MOGPE was then obtained by removing the Fmoc group with piperidine.

Scheme 3 shows the general synthetic scheme used to prepare the anti-HIV lipid containing the phosphatidylcholine headgroup. Briefly, diacylated lecithins were prepared from both the L and D form of glycerophospocholine (L-α-GPC and D-α-GPC) by acylation using 12-MO anhydride. The single chain analog of the L configuration was then prepared from phospholipase A2

cleavage of the diacylated product. For all reactions described above, acylation using the 12-MO-anhydride used 2 equivalents of anhydride per alcohol and 1.5 equivalents of catalysts (DMAP) per alcohol.

CHEMICAL AND SOLVENTS

Chemicals and solvents were used as received unless stated otherwise. 1-myristoyl-sn-glycero-3-phosphoethanolamine (MMPE) was purchased from Avanti Polar Lipis Inc. (Birmingham, AL). L-α-glycerophosphoethanolamine (GPE) and N-(9-Fluorenylmethoxycarbonyloxy) succinimide (Fmoc-NHS) were purchased from Sigma Chemical Company. D-α-glycerophosphocholine was purchased from Biochemisches Labor, Berne CH, Switzerland. Dimethylaminopyridine (DMAP) purchased from Aldrich was crystallized 2 times from ethyl ether. Dicylohexylcarbodiimide (DCC) was purchased from Aldrich. Sodium bicarbonate ($NaHCO_3$) was obtained from Fisher Scientific Chemical Company. Analytical grade chloroform ($CHCl_3$), methanol (MeOH), and tetrahydrofuran (THF) were obtained from Fischer Scientific. H_2O was double distilled from glass containers. Dry THF and Dry $CHCl_3$ were prepared by distillation over calcium hydride. Calcium hydride was purchased from Alpha Products, Danver, MA. 12-methoxydodecanoic acid 12MO was prepared as described hereinabove. 12-methoxyldodecanoyl anhydride (MO-anhydride) was prepared using DCC an purified by crystallization using ethylacetate. Piperidine was obtained from Fisher Scientific Chemical Company. Ninhydrin and Phospray were purchased from Supelco Inc. Bellefonte, PA.

Thin layer chromatography (TLC) was used to monitor all reactions. Silica gel TLC plates were 60 F-254, 0.25 mm thickness (E. Merck, Darmstadt, FGR). Two TLC solvent systems were used: solvent system A contained $CHCl_3/CH_3OH/H_2O$ 65:25:5 V:V:V; solvent B contained $CHCl_3/CH_3OH/H_2O$/THF 64:34:7:30 V:V:V:V. TLC plates were sprayed with either Ninhydrin (Supelco Inc. Bellefonte, PA) to visualize amines or Phospray (Supelco Inc. Bellefont, PA) to visualize phosphate. The extent of reaction was routinely quantified using a scanning densitometer (Shimadzu CS 9000) operating in the reflectance mode. TLC plates were sprayed with Phospray prior to scanning at 600 nm. Phospholipid standards were always included on the same TLC plate used for lipid quantification. Silica gel for flash chromatography was grade 60, 230-400 mesh and obtained from Aldrich Chemical Company. The solvent systems described hereinabove were also used to purify the heteroatom containing phospholipid drugs by flash chromatography.

<u>L-α-glycerophosphoethanolamine-Fluorenylmethyloxycarbonyl (GPE-Fmoc)</u>.

GPE (93 µmole, 20 mg), Fmoc-NHS (130 µmole, 45 mg) and $NaHCO_3$ (288 µmoles, 24 mg) were transferred into a round bottom flask, and 10 mls of $CHCl_3/CH_3OH/H_2O$ (32:17:2 V:V:V) was immediately added to the flask. The reaction mixture was stirred at room temperature. GPE and Fmoc are soluble in this solvent system but $NaHCO_3$ is only slightly soluble. Based on TLC in solvent system A, quantitative yields are obtained in approximately three and half hours. The reaction

mixture was filtered to remove $NaHCO_3$ (solid) and the filtrate was rotoevaporated to dryness. After rotoevaporation the residue was redissolved in $CHCl_3$ (~1 ml) and loaded on to dry silica gel loosely packed in a cylindrical glass frit filtration funnel (~5 g of silica per 1 g of reaction mixture). The unreacted Fmoc-NHS washed off with $CHCl_3$ (10 mls), and NHS washed off with CH_3OH (10 mls). The product was then washed with $CHCl_3/CH_3OH$ 1:1 V:V (10 mls) to obtain pure GPE-Fmoc. GPE-Fmoc (R_f of 0.24) shows one spot on TLC plates developed in solvent system A. GPE-Fmoc is UV positive, Phospray positive, and Ninhydrin negative.

<u>di-(12-methoxydodecanoyl)-sn-glycero-3-phosphoethanolamine-Fluorenylmethyloxycarbonyl (di-12MOGPE-Fmoc)</u>

GPE-Fmoc, MO-anhydride and DMAP were dried in a vacuum desiccator at 40°C for at least four hours before use. GPE-Fmoc (93 µmol, 40 mg), MO-anhydride (410 µmol, 180 mg) and DMAP (200 µmol, 24 mg) were added to a flame dried round bottom flask and freshly distilled $CHCl_3$ (10 mls) was added. The reaction mixture was under a N_2 atmosphere and stirred at 40°C. After 20 hours, TLC in solvent system A confirmed that the reaction was virtually complete; the major product di-12MOGPE-Fmoc had an Rf of 0.46. Rotoevaporation of the reaction solvent left a dry residue which was redissolved in minimal $CHCl_3$ (~1 ml). The $CHCl_3$ solubilized residue was loaded on to silica gel packed inside a cylindrical glass-frit filtration funnel (~5 g silica/g of residue). The unreacted MO-anhydride washed off the silica with $CHCl_3$ (~200 ml); TLC in solvent

system A was used to monitor MO-anhydride in the filtrate. We note that it is important to remove MO-anhydride from the crude product-mixture to avoid decreased retention times and coelution of the products and reactants during column chromatography. After the anhydride was removed, $CHCl_3/CH_3OH/H_2O$ (60:35:5 V:V:V) (~50 mls) was used to wash off the reaction products. Rotoevaporation of the filtrate left a crude product-mixture. The product mixture was redissolved in a minimum volume of mobile phase $CHCl_3/CH_3OH/H_2O$ (65:25:4 V:V:V) and purified using flash chromatography (3 cm x 21 cm column, ~2 mg of reaction mixture per 1 g of silica gel). Fractions eluting from the column (10 ml/fraction) were analyzed by TLC in solvent system A. Fractions containing di-12MOGPE-Fmoc were pooled and the solvent removed by rotoevaporation. The pure product (di-12MOGPE-Fmoc) showed one UV positive TLC spot that was also Ninhydrin negative and Phospray positive. The yield was ~70%.

1,2 di-(12-methoxydodecanoyl)-sn-glycero-3-phosphoethanolamine (di-12MOGPE)

di-12 MOGPE-Fmoc was dissolved in dry $CHCl_3$ (20 mg/ml) at room temperature and piperidine was added [1:80 di-12MOGPE-Fmoc:piperidine]. Fmoc is completely removed in 2 hours but if twice the amount of piperidine is used, then 100% conversion occurs within 45 minutes. On TLC in solvent system A, di-12-MOGPE has an Rf of 0.3 and is both Ninhydrin and Phospray positive but UV negative which indicates that the Fmoc group has been removed. The reaction solvent was removed by rotoevaporation and the crude residue dissolved in 1 ml

of solvent system A and purified by flash chromatography (3 cm x 21 cm) using the same solvent system. Fractions were collected (10 ml/fractions) and analyzed by TLC in solvent system A. Fractions containing the product were pooled and the solvent removed by rotoevaporation to obtain pure di-12MOGPE. The final lecithin product (di-12MOGPE) exhibited one spot on TLC in solvent system A and was Ninhydrin positive, Phospray positive, and UV negative.

FAB-MS: MH^+ 640.3. IR (CaF_2, neat) υ_{as} CH_2 2917.4; υ CH_2 2850.4; υ C=O 1738.4; δ_{as} CH_2 1454.0; υas PO_2 1230.6; υC-O-C 1077.8; υ_s PO_2 1027.9. ^1H NMR (500 MHz, $CDCl_3$) results: δ 5.18 ppm (br s, 1H, CH), 4.35 ppm (m, 1H, CH_2OP), 4.11 ppm (m, 1H, CH_2OP), 4.05 ppm (br s, 2H, CH_2OP), 3.90 ppm (br s, 2H, CH_2OCO), 3.33 ppm (t, 4H, OCH_2), 3.30 ppm (s, 6H, OCH_3), 3.12 ppm (br s, 2H, NCH_2), 2.28 ppm (m, 4H CH_2COO), 1.55 ppm (m, 8H $\underline{CH_2}CH_2COO$, $\underline{CH_2}CH_2OCH_3$), 1.25 ppm (br s, 28H, $(CH_2)_7$).

<u>1,3 di-(12-methoxydodecanoyl)-sn-glycero-2-phosphoethanolamine-Fluorenylmethyl-oxycarbonyl (1,3 di-12MOGPE-Fmoc)</u>

Headgroup migration occurred during the preparation of di-12MOGPE-Fmoc and the migration product was 1,3 di-12 MOGPE-Fmoc. This migration product was purified by flash chromatography as described above for di-12MOGPE-Fmoc. 1,3 di-12MOGPE-Fmoc exhibited one spot on TLC in solvent system A with an Rf = 0.4.

^1H NMR (500 MHz, $CDCl_3/CD_3OD$) results: δ 7.68 ppm (d, 2H, aromatic), 7.55 ppm (d, 2H, aromatic), 7.30 ppm (t, 2H, aromatic), 7.22 ppm (t, 2H, aromatic), 4.33 ppm (br s, 1H, CHOP), 4.28 ppm (d, 2H, CH_2CHCC), 4.10

ppm (t, 1H, CHCC), 4.15 ppm (m, 1H CH_2OP), 3.90 ppm (m, 1H, CH_2OP), 3.82 ppm (br s, 2H, CH_2OP), 3.80 ppm (br s, 2H, CH_2OCO), 3.42 ppm (br s, 2H, NCH_2), 3.30 ppm (t, 4H, OCH_2), 3.23 ppm (s, 6H OCH_3), 2.15 ppm (t, 4H, CH_2COO), 1.50 ppm (m, 8H CH_2CH_2COO, $CH_2CH_2OCH_3$), 1.20 ppm (br s, 28H, $(CH_2)_7$).

1-myristoyl-sn-glycero-3-phosphoethanolamine-Fluorenylmethyl-oxycarbonyl (MMPE-Fmoc)

MMPE (9.88 mmoles, 4.2 g) and insoluble $NaHCO_3$ (29 mmoles, 2.4 g) were mixed in 50 mls of $CHCl_3/CH_3OH/H_2O$ 32:17:4 V:V:V for 2 minutes at room temperature prior to the addition of Fmoc-NHS (13.3 mmoles, 4.5 g). After 2-3 hours the reaction was virtually complete based on TLC in solvent system A. Without $NaHCO_3$ the yield was always 50-60% regardless of reaction conditions. The reaction was filtered through a fine glass-frit funnel to remove $NaHCO_3$ (solid) and the filtrate was rotoevaporated to obtain a residue. The dry residue (~9 g) was redissolved in minimum $CHCl_3$ (2-3 ml) and loaded on to dry silica loosely packed in a glass filtration funnel (~5 g of the silica gel per g of reaction mixture). Based on TLC in solvent system A, unreacted Fmoc-NHS washed off the silica with $CHCl_3$ (~100 mls/g-product). After removing Fmoc-NHS from the product-mixture, both the phospholipid-product and NHS byproduct coeluted using ~300 mls of $CHCl_3/CH_3OH/H_2O$ (32:17:4 V:V:V). The mixed solvent containing the product was removed by rotoevaporation and the residue extracted with $CHCl_3/CH_3OH/H_2O$ 8:4:3 V:V:V to remove NHS and other impurities. The product remained in the organic phase during the extraction. During extraction

approximately 20% of the product was lost into the aqueous phase but was recovered by reextraction of the aqueous phase with fresh organic solvent. MMPE-Fmoc was an amorphous white solid after lyophilization from benzene. The pure MMPE-Fmoc shows one spot (Rf of 0.36) during TLC in solvent system A. MMPE-Fmoc is UV positive, Phospray positive and Ninhydring negative. Product yields ar 70-90% based on 2 reactions.

IR (CaF_2, neat) results: OH 3336.0 (broad); υ_{as} CH_3 3064.7; υ_s CH_3 2953.1, υ_{as} CH_2 2923.9; υ_s CH_2 2852.9; υC=O 1721.7; υ1533.8; δ_{as} CH_2 1450.3; υ_{as} PO_2 1236.2; υC-O-C 1108.5; υ_s PO_2 1069.0. ^1H NMR (500 MHz, $CDCl_3/CD_3OD$) results: δ7.55 ppm (d, 2H, aromatic), 7.40 ppm (d, 2H, aromatic), 7.18 ppm (t, 2H, aromatic), 7.10 ppm (t,2H, aromatic), 4.15 ppm (m, 1H, CH_2OP), 3.95 ppm (t, 1H, CHCC), 3.90 ppm (d, 2H, CH_2CHCC), 3.75 ppm (m, 1H, CH_2OP), 2.70 ppm (br s, 2H, CH_2OP), 3.65 ppm (br s, 2H, CH_2OCO), 3,18 ppm (t, 2H, NCH_2), 2.08 ppm (t, 2H, CH_2COO), 1.35 ppm (m, 2H CH_2CH_2COO), 1.05 ppm (br s, 20H, $(CH_2)_{10}$), 0.70 ppm (t, 3H, CH_3).

<u>1-myristoyl-2-[12-methoxydodecanoyl]-sn-glycero-3-phosphoethanol-amine-Fluorenylmethyloxycarbonyl (1-M-2-12 MOGPE-Fmoc).</u>

MMPE-Fmoc, MO-anhydride and DMAP were dried in a 45°C vacuum desiccator for at least 4 hours. MMPE-Fmoc (154 µmol, 100 mg) was dissolved in freshly distilled THF in a flame dried round bottom flask, and 12-MO-anhydride (632 µmole, 280 mg) and DMAP (460 µmol, 56 mg) were also dissolved in distilled THF but in a separate flask. Both flasks were heated to 45°C and after the reactants dissolved, MO-anhydride and DMAP

were carefully transferred to the flask containing MMPE-Fmoc (20 mg reactant/ml solvent). The reaction mixture was purged with nitrogen and stirred. After 1 hour the reaction was cooled to room temperature and allowed to react for another 14 hours. The solvent was removed by rotoevaporation and a minimum volume of $CHCl_3$ (~1-2 ml) was used to dissolve the residue. Unreacted MO-anhydride was removed from the reaction mixture and the product was purified as described above for di-12MOGPE-Fmoc with the minor modification that the mobile phase solvent was solvent system B. The purified product (1-M-2-12MOGPE-Fmoc) exhibited one spot (Rf = 0.48) on TLC plates developed in solvent system B. 1-M-2-12MOGPE-Fmoc was Ninhydrin negative, phospray positive and UV positive. The yield was ~92% based on 1 reaction.

1-myristoyl-2-[12-methoxydodecanoyl]-sn-glycero-3-phosphoethanolamine (1-M-2-12MOGPE)

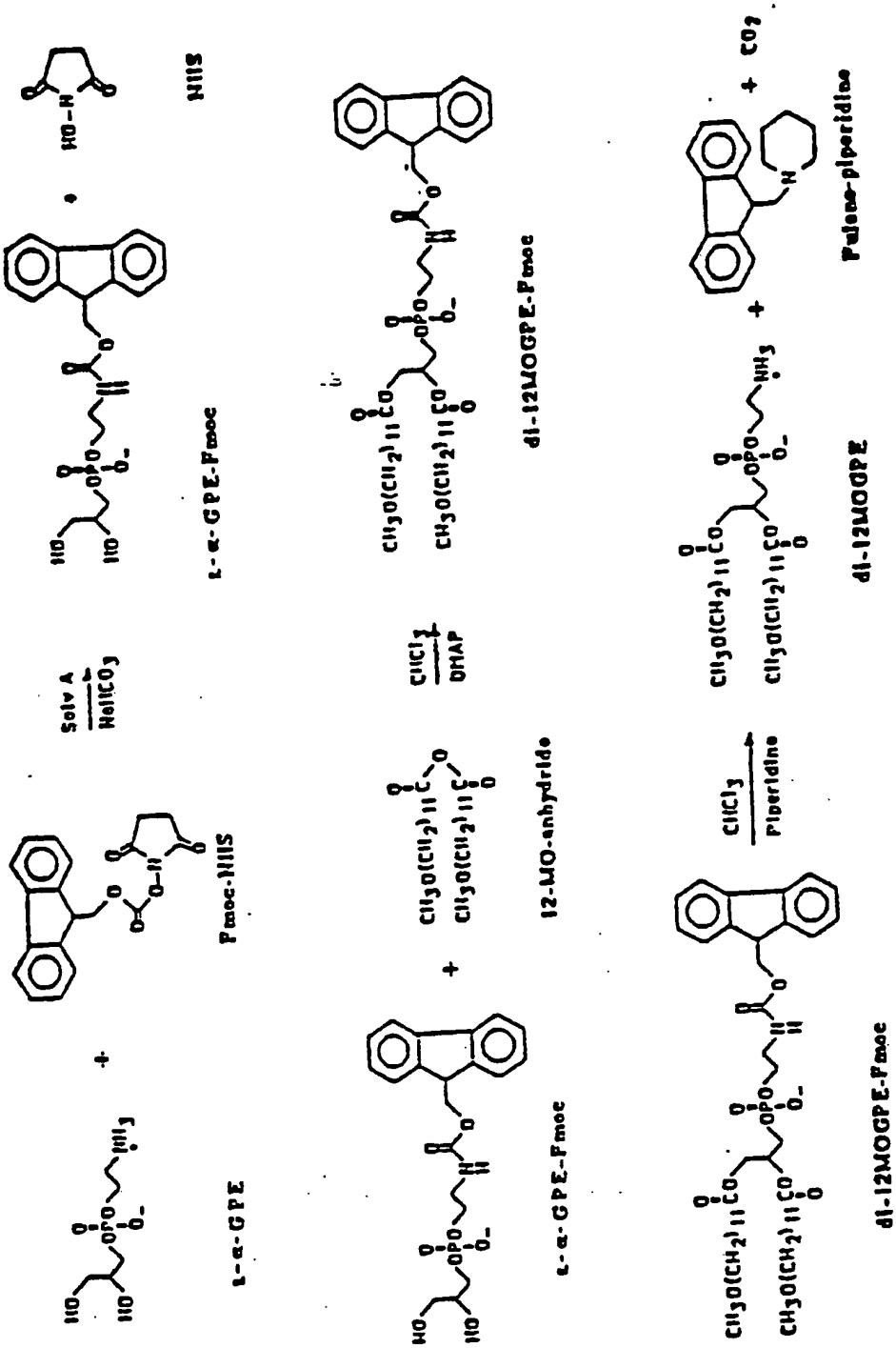
FMOC was removed from 1-M-2-12MOGPE-Fmoc by piperidine and the lecithin product (1-M-2-12MOGPE) purified by flash chromatography as described above for di-12MOGPE. Similar to purification of di-12MOGPE, the purification of 1-M-2-12MOGPE was simple because Fmoc elutes at the solvent front whereas piperidine remains at the origin when $CHCl_3/CH_3OH/H_2O$/THF (64:34:7:30) is used as an isocratic solvent system during flash chromatography. The final product, 1-M-2-12 MOGPE, shows one spot on the TLC plates developed in solvent system A. 1-M-2-12-MOGPE is UV negative, and positive when sprayed with either Ninhydrin or Phospray. The yield was ~84 % based on 1 reaction.

FAB-MS: MH^+ 638.5. IR (CaF_2, neat) results: υ_{as} CH_2 2918.3; υ_s $CH_2$2850.6; υ C=O 1739.0; δ_{as} CH_2 1467.4; υ_{as} PO_2 1230.8; υ C-O-C 1078.8; $\upsilon_s PO_2$ 1028.2. ^1H NMR (500 MHz, $CDCl_3$), δ 5.18 ppm (br s, 1H, CH), 4.35 ppm (m, 1H, CH_2OP), 4.11 ppm (m, 1H, CH_2OP), 4.05 ppm (b s, 2H, CH_2OP), 3.90 ppm (br s, CH_2OCO), 3.33 ppm (t, 2H, OCH_2), 3.30 ppm (s, 3 H, OCH_3), 3.12 ppm (br s, 2H, NCH_2), 2,28 ppm (m, 4H, CH_2COO), 1.55 ppm (m, 6H CH_2CH_2COO, $CH_2CH_2OCH_3$), 1.25 ppm (br s, 34 H, $(CH_2)_7$, $(CH_2)_{10}$), 0.85 ppm (t, 3H, CH_3).

Preparation of DAC2, LPE1 and LPE2

The preparation of D-AC2 was identical to L-AC2 except D-glycerolphosphocholine, which was obtained from Synthetische Phosphor-Lipide, Biochemisches Labor, Bern CH Switzerland was used. L-PE2 was prepared by reacting glycerolphosphatidylethanolamine (L-a-GPE) with N-(9-Fluorenylmethoxycarbonyloxy) succinimide (FMOC-succinimide) to form L-a-GPE-FMOC followed by diacylation with 12MO anhydride then deprotection of FMOC with piperidine. L-PE1 was prepared by diacylating L-a-GPE-FMOC with myristic anhydride, then PLA2 cleavage to remove the sn-2 chain, then acylation with 12MO anhydride, and finally deprotection with piperidine to remove FMOC.

Scheme 1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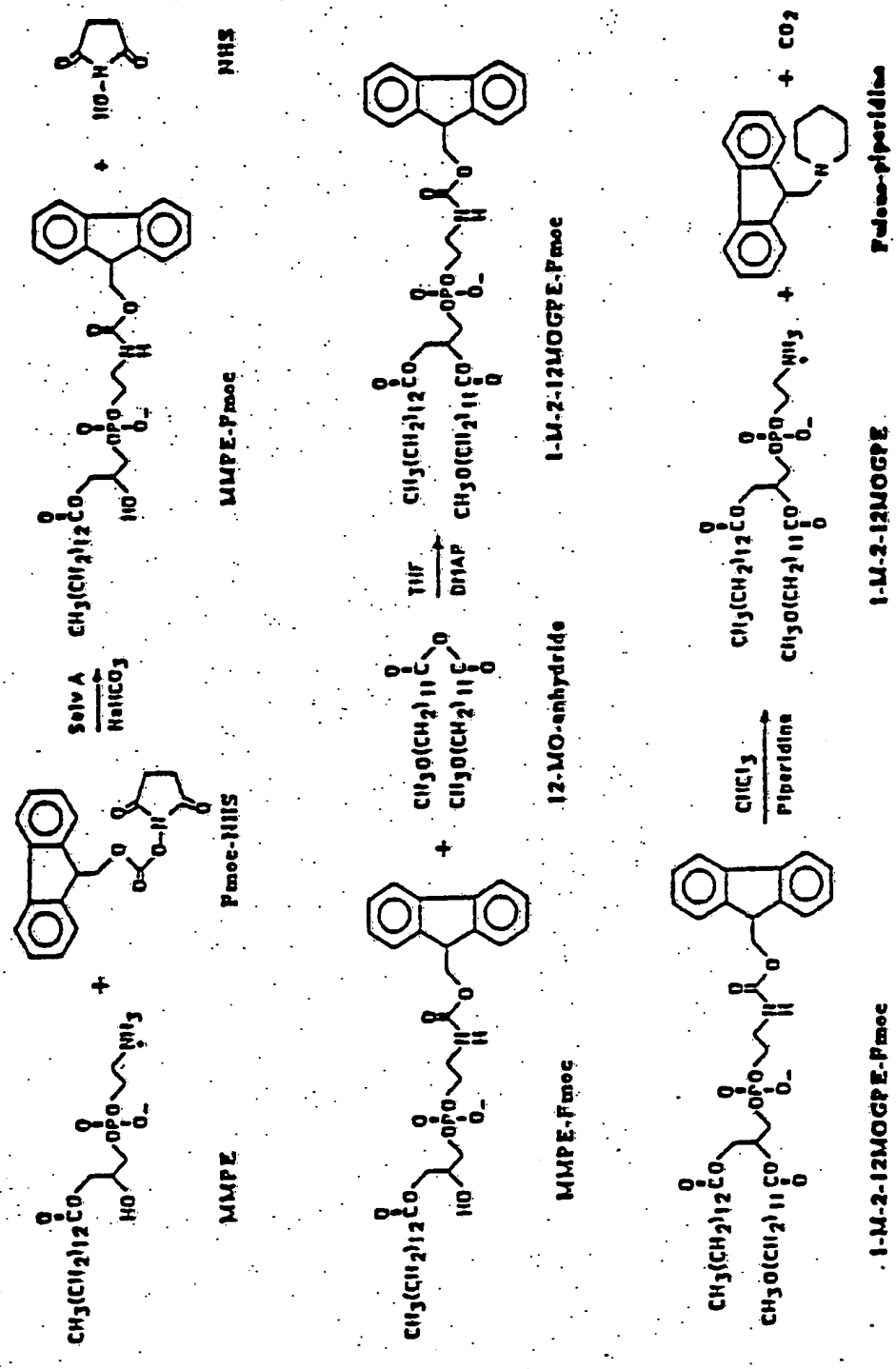
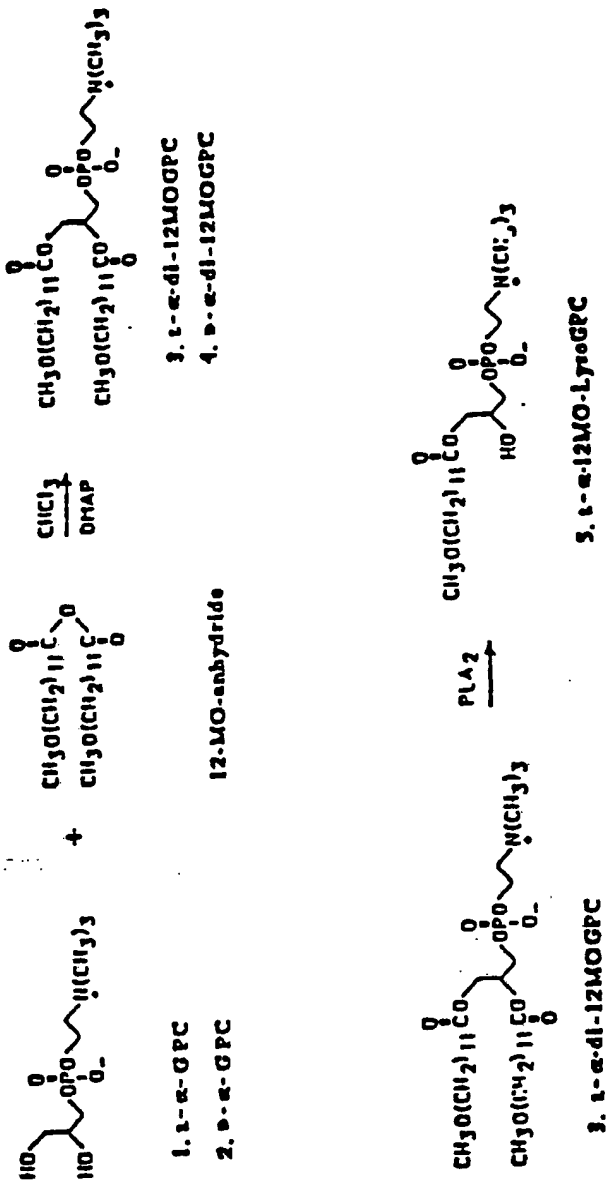
di-12MOGPE-Fmoc
Fulene-piperidine
+ CO2
Solv A
NaHCO3
ClHCl3
DMAP
ClHCl3
Piperidine
Fmoc-NHS
12-MO-anhydride
L-α-GPE
L-α-GPE-Fmoc
di-12MOGPE
di-12MOGPE-Fmoc

Scheme 2


NHS
MMPE-Pmoc
Solv A
NaHCO3
Pmoc-NHS
MMPE
THF
DMAP
1-M-2-12MOGPE-Pmoc
12-MO-anhydride
MMPE-Pmoc
ClHCl3
Piperidine
Pmoc-piperidine
1-M-2-12MOGPE
1-M-2-12MOGPE-Pmoc

Scheme 3


ClCl3
DMAP
PLA2
1. 1-α-GPC
2. 2-α-GPC
12-MO-anhydride
3. 1-α-di-12MOGPC
4. 2-α-di-12MOGPC
3. 1-α-12MO-LysoGPC

Single chain phospholipid analogs are known to form micelles, whereas double chain analogs form liposomes. After injection into animals or man, liposomes concentrate in macrophages, yet micelles do not. Thus, phospholipid analogs containing biologically active fatty acid molecules in the alkyl chains can be modified such that the dispersion properties of the phospholipids can be used to control, in part, the in vivo disposition of these anti-HIV drugs. Macrophages and T-cells are both CD4 positive cells and consequently, HIV avidly infects both of these cells. Thus, liposome forming anti-HIV drugs have unique application for combination therapy against HIV, particularly during viremia. If inhibitors of the HIV-CD4 binding interaction are found and employed for HIV therapy, then during viremia, HIV may not concentrate in T-Cells. However, this may cause the HIV infection to shift to other cells such as blood monocytes and macrophages, which internalize foreign particles regardless of the presence of CD4. The ability to control the in vivo disposition of, for e.g., phosphatidylcholine anti-HIV compounds, by changing the number of acyl chains on the phospholipids, is therefore significant.

It is believed that liposome forming anti-HIV active phospholipids will most likely not be able to treat HIV infected tissue macrophages because liposomes cannot efficiently exit the blood compartment of the host. However, single chain lysolipid analogs which form micelles are expected to distribute into tissues outside of the blood compartment since lysolipid analogs

bind to albumin and are transported by albumin to different tissues.

The incorporation of biologically active fatty acids themselves into liposomes, for delivery to mononuclear phagocytic cells, will also concentrate the drug in blood monocytes/macrophages. However, no control over the incorporation of hetero-atom fatty acids into the cellular lipid pool exists for this delivery system. In contrast, feeding cells biologically active fatty acids covalently linked to particular phospholipids allows some control over the cellular disposition of the biologically active fatty acids. Thus a key concept for the delivery of biologically active fatty acids in the form of phospholipids is that the in vivo disposition of the drugs can be controlled by liposome forming analogs and the cellular disposition of biologically active fatty acids can be altered for therapeutic benefit by using specific phospholipid headgroups for the preparation of the drugs.

I. Experimental Designs and Methods

Overview of assay methods

Three assays for anti-HIV activity were used and the IC_{50} was calculated from these assays; (i) syncytial cell assays, (ii) reverse transcriptase assays, and (iii) direct cytotoxicity assays. In addition, direct drug cytotoxicity was tested 'as a control' in each assay which merely means that the drug was dosed to the cells without virus present. This control assured that only the antiviral effect of the compound is measured. However, this drug cytotoxicity

was not used to calculate the TC_{50} because the TC_{50} required doses of drug that were higher than the effective concentrations. Thus the TC_{50} is the dose of drug that kills 50% of the cells without virus present and was measured in MTT assays using the same cells type to evaluate antiviral activity. The therapeutic index was calculated from TC_{50}/IC_{50}. The ability of anti-HIV phospholipids to inhibit direct cytopathic effect caused by HIV infection was also measured.

Anti-HIV activity of AC2, AC1, lysoAC2 and 12MO

Viral stocks

The S5G7 strain of HIV was used. S5G7 is a subclone of HTLVIIIB grown in H9 cells, and highly virulent regarding T cell infectivity, but less so for monocytes; this strain was obtained from Abbott Laboratories.

Anti-HIV activity: (1) direct cytotoxicity assay

This assay measures the ability of a drug to inhibit the HIV virus from killing cells. CEM cells were used for this assay. The protocol is given in the next two paragraphs.

The T-cell HIV inhibition assay method of acutely infected cells is an automated tetrazolium based colorimetric assay adapted from Novak, et al., Aids Res. and Human Retroviruses, 6, 973 (1990). Assays were performed in 96-well tissue culture plates. CEM cells were grown in RPMI-1640 medium (Gibco) supplemented with 10% fetal calf serum and were then treated with polybrene (2 µg/ml). A 80 µl volume of medium containing 1 x 10^4 cells was dispensed into each well of

the tissue culture plate. To each well was added a 100 µl volume of test compound dissolved in tissue culture medium (or medium without test compound as a control) to achieve the desired final concentration and the cells were incubated at 37°C for 1 hour. A frozen culture of HIV-1 was diluted in culture medium to a concentration of 5 x 10^6 $TCID_{50}$ per ml ($TCID_{50}$ = the dose of virus that infects 50% of cells in tissue culture), and a 20 µl volume of the virus sample (containing 1000 $TCID_{50}$ of virus) was added to wells containing test compound and to wells containing only medium (infected control cells). This results in a multiplicity of infection of 0.1 (MOI = # of infectious virus particles/# of cells in culture). Several wells received culture medium without virus (uninfected control cells). Azidothymidine (AZT) was tested as a positive drug control. Test compounds were dissolved in DMSO and diluted into tissue culture medium so that the final DMSO concentration did not exceed 1.5%. DMSO had no significant effect on results as determined in controls.

Following the addition of virus, cells were incubated at 37°C in a humidified, 5% CO_2 atmosphere for 7 days. Additional aliquots of test compounds were added on days 2 and 5. On day 7 post-infection, the cells in each well were resuspended and a 100 µl sample of each cell suspension was removed for assay. A 20 µl volume of a 5 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each 100 µl cell suspension, and the cells were incubated for 4 hours at 37°C in 5% CO_2 environment. During this incubation, MTT is metabolically reduced by living cells resulting in the production in the cell of

colored formazan product. To each sample was added 10 ml of 10% sodium dodecylsulfate in 0.01N HCl to lyse the cells, and samples were incubated overnight. The absorbance of each sample was determined at 590 nm using a Molecular Devices microplate reader. The % reduction of the virus induced cytopathic effect (CPE) by the test compounds was calculated from the equation.

$$\% \text{ reduction CPE} = \frac{(\text{Abs compound-treated infected sample}) - (\text{Abs of virus control})}{(\text{Abs of cell control}) - (\text{Abs of virus control})} \times 100$$

The direct cytotoxicity of each compound to CEM cells is labeled above each histogram bar in Figure 1. Starting from the top graph in Figure 1, AC1 was not toxic to the CEM cells at dose up to 400 µM, AC2 was toxic to CEM cells at 400 µM, lysoAC2 was not toxic up to 400 µM, and 12MO was toxic to CEM cells at 400 µM but 12MO also showed toxicity at 40 µM. 12MO was the most toxic analog tested in this series.

The IC_{50} of AC2 could not be accurately determined from the data because the activity was too high but the IC_{50} is less than 4 µMolar, and it is estimated that the IC_{50} is ~1 µM. It is also interesting that anchoring the biologically active fatty acid in the sn-2 position significantly reduced the activity (i.e., AC1 top graph), but the lysolecithin analog containing the biologically active fatty acid in the glycero sn-1 position was active with an IC_{50} ~100 µMolar. This may be due to the metabolism of lysolipids compared to diacylated lipids or it may indicate the myristoyl group is stored in the sn-1 chain of endogenous membrane lipids. AC1 and the lyso compound

were significantly less toxic than the biologically active fatty acid of AC2.

Anti-HIV activity: (2) macrophages

Anti-HIV activity was next measured in macrophages using a p24 antigen capture assay. The assay protocol is outlined below.

Absorbance values (492 nm) for HIV-1 p24 antigen were detected by enzyme-immunoassay (EIA) in culture supernatants of HIV-1 (3B) infected monocyte-derived macrophages (MDM). Human peripheral blood MDM were prepared by adherence to plastic in 24-well tissue culture plates (Costar, Cambridge, MA). Briefly, 1 x 10^7 Ficoll-Paque (Pharmacia, Piscataway, NJ) gradient purified mononuclear cells in RPMI-1640 with 20% heat-inactivated fetal bovine serum, L-glutamine, and gentamycin (Gibco, Grand Island, NY) were placed in each well and allowed to adhere at 37°C for 3 hours. Non-adherent cells were removed by gentle washing with warm (37°C) Hank's balanced saline solution (HBSS-Gibco) and the cells incubated in a 5% CO_2 in air atmosphere at 37°C in 2 ml of media. Remaining non-adherent cells were further removed by washing again after 24 hours and 5 days. After 7 days in culture, the cells were infected with HIV-1 3B by removing the media from each well, washing with HBSS and adding 0.2 ml of virus-containing supernatant from a 5 day culture of a MDM permissive subclone of HIV-1 3B grown in H9 cells. The plate was rocked at 37°C for 3.5 hr, the viral inoculum removed and the cells washed 3 times each with 2 ml of warm HBSS to remove non-MDM-associated viral particles. Media containing 0, 1, 10, 50 or 100 µM of AC1, AC2 or

12MO were added to the appropriate wells and the cells incubated as previously described. On days 1, 3, 6, 8, 10 and 15 after infection, 200 µl of supernatants were removed from each well for p24 antigen EIA assay (HIVAG-1; Abbott Laboratories, North Chicago, IL). On day 8, following the sampling for p24 antigen, 1 ml of fresh media containing AC1, AC2, or MO was added to the appropriate wells to restore a concentration of 1, 10, 50 or 100 µM of each compound. Data is presented as absorbance values reflecting HIV-1 p24 antigen concentration produced by infected MDM as detected in culture supernatants. The experiment shown in Figure 2 is representative of 3 experiments using 3 cell donors. In Figure 2 the dose of drug is given above each bar in the histogram, and -C is the negative control (no virus, no drug).

Figure 2 shows that AC2 was very potent and completely inhibited the AIDS infection in macrophages at all doses tested. Even at the low dose of AC2, i.e., 1 µM, AC2 was completely effective. AC1 gave a dose response with almost complete inhibition at 100 µM. 12MO exhibited little activity except at 100 µM where the HIV infection was completely suppressed. At this concentration, however, 12MO was toxic.

<u>Anti-HIV activity: (3) Syncytial cell assays</u>

Briefly, the assay was performed as follows. On Day 0, MT4 cells were infected with HIV. On Day 3, mix 10^3-10^4 infected cells (resuspend cells at 10^4-10^5 cells/ml so that the desired number of cells is in a volume of 100 µl) with 10^5 SupT1 cells (suspended at

10^6/ml, so that there are 10^5 cells per 100 µl) in a 96 well plate. Infected cells are titered to find the optimum number of syncytial cells to count. Cells are counted either manually or by flow cytometry. Syncytia begin to form at 8-10 hours, but optimum time for MT4 cells is about 18 hours. The time intervals for other cell lines may vary. To assay drugs that block syncytia formation, 50 µl SupT1, 50 µl infected (MT4) cells (total cell number is the same), and 100 µl of drug are present. Typical results are shown in Figure 3 which compares the IC_{50} of AC2 to 12MO. The IC_{50} of AC2 is ~1 µM and the IC_{50} of 12MO is ~4 µM.

Antiviral Activity in PHA-Lymphoblasts: (4) Reverse Transcriptase Assay

Mononuclear cells were obtained from the whole blood of normal donors by ficoll/Hypaque (Pharmacia) density gradient centrifugation. These cells are initially washed with buffer and then stimulated with PHA-M (Gibco) for 72 hours. The cells are then counted for number, and also viability using trypan blue exclusion, followed by infection with S5G7, a subclone of HTLVIIIB or a wild type strain, at a multiplicity of infection (MOI) of 0.2 (i.e., 1 virus/5 cells) in a volume of 0.2 ml of culture supernatants for 2 hours at 37°C. Control cells not challenged with virus are used to evaluate drug toxicity. Cells are then washed three times in RPMI + 10% fetal calf serum (FCS) and plated in a 96-well tissue culture plate in RPMI + 10% FCS, 10mM L-GLN, 10mM Sodium pyruvate, IL-2, gentamicin. Cells are refluxed every 24 hours with complete media containing replacement drug and IL-2. Seven days after

infection, samples are taken for the reverse transcriptase assay. These samples are frozen at -70°C until analysis.

The procedure for measuring RT (reverse transcriptase) activity can be routinely performed by one of ordinary skill. Briefly, 50 µl of HIV culture supernatant are mixed with 50 µl of a 2X RT assay buffer containing Tris, 0.1M, pH 7.9, KCl, 0.32M, dithiothreitol, 0.012M, $MgCl_2$, 0.012M, reduced glutathione, 1.2 mM, ethylene glycol-bis(beta-aminoethylether)-N,N,N',N'-tetraacetic acid, 1mM, ethylene glycol, 4%, sterile, distilled water, 10 µl, Triton X-100, 0.2%, template primer poly(rA).p(dT), 1 µ/ml, 0.05 µ/sample, (Pharmacia), and 10 µCi ^{3}H-dTTP (Amersham). Samples are incubated for 24 hours at 37°C in microtiter plates, after which the reaction is stopped with 200 µl cold 10% tetrachloroacetic acid containing 0.2 M sodium PPi. The plate is then allowed to incubate for 2 hours on ice, after which, samples are harvested onto DE-81 filter paper discs (Whatman) using a cell harvester. The discs are washed 8 times in 5% trichloroacetic acid and absolute ethanol, dried and placed into glass scintillation vials. They are then counted on a beta scintillation counter. Negative (uninfected cell supernatants are used to determine the background DNA polymerase activity, if any) and known positive controls are assayed simultaneously. Results measured in counts per minute (cpm) are plotted as % of the control (i.e., cpm obtained without drug present but with virus infection) as shown in Figure 4.

Figure 4 shows that the IC50 of AC2 is ~4 μM and the IC50 of 12MO is ~12 μM in this reverse transcriptase assay.

Drug cytotoxicity using MTT assay (calculation of TC_{50})

MT4 cells were plated in 96-well microtiter plates at 8 x 10^4 cells/well in a volume of 90 μl. [Note: To compare TC50 to IC50, the same cell type used in antiviral assays was tested in the MTT assay; for these experiments, MT4 cells were used to evaluate the syncytial cell assays and PBMC's were used to compare the PBMC/RT assay]. To this was added one minimum cytotoxic dose of HIV in a volume of 10 μl. The test drug was added in 100 μl aliquots at several concentrations. A control plate using uninfected MT4 cells was set up simultaneously to assess cytotoxicity due to the drug alone. The plates were then incubated for five days at 37°C. Then the media was aspirated from the wells and replaced with 100 μl MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 1mg/ml in PBS) and incubated for 4 hours at 37°C. The plates were then centrifuged to pellet the cells, and the supernatants were removed (centrifugation can be omitted if the supernatants are removed carefully). Acidic isopropanol (0.04N HCl in isopropanol), 100 μl, was then added to the wells and shaken for 15-30 minutes to dissolve the formazan crystals. Plates were read on an ELISA reader at Abs 570 nm. The difference between the uninfected and infected plates represent the antiviral activity of the drug. This assay was used to determine drug toxicity alone. A similar method has

been described in the AIDS Research and Reference Reagent Program Courier, 90-01:8-9, 1990.

Figure 5 shows the results of a typical experiment run in MT 4 cells which can be compared directly to the syncytial cell assay shown in Figure 3 because the same cell line was used. The TC50 of AC2 was 280 µM and the TC50 of 12MO was 140 µM as shown in Figure 5.

Therapeutic Index of Lipids having anti-HIV-Activity

Table 1 summarizes the data shown in Figure 1-5 by comparing the therapeutic index for 12MO and AC2. The therapeutic index compares the toxic dose (TC50) to the effective dose (IC50). The therapeutic index values given in Table 1 are calculated from toxicity data and activity data that were obtained in the same cell line. In syncytial cell assays using MT 4 cells the therapeutic index for AC2 is 280 whereas for 12MO the therapeutic index is only 35. In PBMC's, the therapeutic index for AC2 is 31 whereas for 12 MO the therapeutic index is 37.

Moreover, L-AC2 was significantly more potent than 12MO in HIV infected monocyte-derived macrophages (MDM) shown in Figure 1. During a 15 day acute infection, L-AC2 at 1 µM completely suppressed the HIV infection in MDM yet 12MO had little activity at doses from 1 to 50 µM. However, the 100µM dose of 12MO suppressed HIV replication in MDM which demonstrates that 12MO exhibited a very steep dose response effect in MDM i.e., little activity at 50µM and virtually 100% activity at 100 µM. No dose response was observed in

MDM for L-AC2 because the lowest dose of L-AC2 tested (1 μM) completely inhibited the HIV infection in MDM.

In HIV infected MDM, L-AC1 exhibited dose responsive activity and 50 μM L-AC1 completely suppressed HIV p24 antigen production; this dose of L-AC1 had little activity in CEM cells using a direct cytopathic assay (Figure 1). Diacylated phospholipids form liposomes, and as expected both L-AC1 and L-AC2 formed liposomes in aqueous buffers. The increased activity of L-AC1 in MDM compared to CEM cells may be due to the phagocytosis of L-AC1 liposomes in MDM.

Table I. IC_{50} and Therapeutic Index of L-AC2 and 12MO

	TC_{50}	IC_{50} [b]	Therapeutic Index[c]	IC_{50} [b]	Therapeutic Index[c]
	Cytotoxicity[a] MTT Assay	50% Reduction in syncytial cell formation MT4 cells[d]	MT4 cells	50% Reduction in Reverse Transcriptase Activity PBMC[e]	PBMC
	µM	µM		µM	
12 MO	140 (MT4 cells)	4	35		
	340 (PBMC cells)			19	17
L-AC2	280 (MT4 cells)	1	280		
	220 (PBMC cells)			6	37

a) Drug cytotoxicity in the absence of HIV was determined using MT4 cells or PBMC's. The TC_{50} is the concentration of the frug that killed 50% of the cells during a 4 hour incubation period.

b) The IC_{50} is the drug concentration that inhibited 50% of the maximum HIV response which was observed when no drug was present. The HIV responses that were measured are syncytial cell formation of reverse transcriptase activity.

c) The therapeutic index was calculated as TC_{50}/IC_{50}.

d) Syncytial cell assays were performed using MT4 cells infected with HIV and SupT1 cells as target cells. MT4 cells are CD4+ and highly susceptible to HIV infection.

e) Drug activity was determined in PBMC by measuring inhibition of reverse transcriptase activity.

Compounds of the present invention (IV, V, VI), especially those containing at least one heteroatom fatty acid acyl chain, exhibit synergistic effects when administered with AZT. The more preferred embodiments (compounds of formulae VIII-XV) also exhibit this effect. An illustrative example is given hereinbelow.

Synergism of AC2 with AZT

Another set of data demonstrates the synergism observed with AC2 and AZT in syncytial cell assays using T cells.

12MO has been reported to act synergistically when administered concurrently with AZT, and the synergism between AZT and either 12MO or L-AC2 using syncytial cell assays was evaluated. (Figure 6). The dose response curves for each drug alone shows that AZT is ~100 times more potent than L-AC2; however, AZT is ~10^3 to 10^4 times more potent than 12MO. Synergism was

evaluated by the shift in the dose response curves when 0.5 nM AZT (an inactive concentration) was added to 12MO and L-AC2; 12MO exhibited less than a factor of 10 increase in activity whereas L-AC2 exhibited approximately 100 fold increased activity (Figure 6). For instance, Figure 6 shows that 5 nM L-AC2 (an inactive concentration labelled ** in Figure 6) and 0.5 nM AZT (an inactive concentration) exhibited ~50% inhibition of syncytia formation in HIV infected MT4 cells. Figure 6 also shows that 10 nM L-AC2 (an inactive concentration) shifts the dose response curve of AZT approximately 100 fold. Using MT4 cells, concentrations of AZT from 50 nM to 500 nM did not alter the concentration of L-AC2 that killed 50% of uninfected cells, i.e., the TC_{50} of L-AC2 is unchanged in the presence of AZT.

Figure 6 clearly shows the increased synergistic effects of L-AC2 with AZT as compared with 12MO and AZT. Without wishing to be bound, it is believed that the synergism for L-AC2 and AZT is not due to increased cellular toxicity from administering both drugs concurrently; it is believed that the synergism is due to direct inhibition of HIV by two different mechanisms. AZT is a reverse transcriptase inhibitor; whereas, L-AC2 putatively inhibits endogenous myristoylation of the HIV proteins.

Anti-HIV Activity of D-AC2

L-AC2 contains the natural configuration of glycerophosphocholine and is quantitatively hydrolyzed by bee venom phospholipase A2 (PLA2) within minutes. PLA2s stereospecifically hydrolyze phospholipids.

However, the D-isomer, i.e., D-AC2 was prepared to test the hypothesis whether endogenous PLA2s are responsible for 12MO release from phosphatidylcholine analogs containing 12MO. Figure 7 shows that the IC_{50} for D-AC2 is ~1 uMolar which is identical to the IC_{50} of L-AC2. Unlike L-AC_2, D-AC2 is not hydrolyzed by PLA2.

Figure 7 also shows the anti-HIV activity of the phosphatidylethanolamine (PE) analogs L-PE1 and L-PE2; these analogs are chemically similar to L-AC1 and L-AC2 respectively except the PC headgroup has been changed to the PE headgroup. The IC_{50} of L-PE1 and L-PE2 are 6 uMolar and 0.02 uMolar respectively; compared to the PC analogs this is approximately a 20-50 fold increase in activity. The anti-HIV activity of L-PE2 is > 100 fold more than 12MO (Figure 7).

Stability of Anti-HIV Phospholipids in Fresh Blood at 38°C

Drug development using phospholipids will require that the parent compound is stable in blood. Figure 8 shows that the halflife of L-AC2 in fresh blood is 4.56 hours and the halflife of D-AC2 is 18.24 hours (~4 times longer). Thus, by changing the stereochemistry of the glycerobackbone to the unnatural configuration the halflife in blood can significantly be increased. Changing the lipid headgroup also increases the stability in blood. L-PE2 has a halflife in fresh blood of 9.36 hours which is approximately 2 times longer than L-AC2 (Figure 8). It was very surprising that L-PE1 has a very long halflife ($T_{\frac{1}{2}}$ > 50 hours) compared to the L-PE2. L-PE1 and L-PE2 are identical except that the methylene group in the 13 position of

the sn-2 alkyl chain has been replaced with an oxygen atom.

Without wishing to be bound, it is believed that the increased activity, particularly of L-AC2 and L-PE2 compared to 12MO, and also the increased synergism of L-AC2 with AZT compared to 12MO and AZT is due to the cellular disposition of phospholipid analogs. When 12MO is delivered to cells as a free fatty acid it is rapidly incorporated into triglycerides and membrane lipids. The $T_{\frac{1}{2}}$ for incorporation is approximately 1-2 minutes. Triglycerides are usually thought of as storage depot for fats that are used as a source of energy. If intracellular triglycerides containing 12MO are used primarily as an energy source instead of a source of fatty acids for myristoylation of HIV proteins, then this may be the primary reason why the cellular availability, necessary for anti-HIV activity, of 12MO is 10 fold or 100 fold less than L-AC2 and L-PE2. Thus lipid metabolism and the intracellular disposition of hetero atom fatty acids and anti-HIV phospholipids can significantly affect anti-HIV activity.

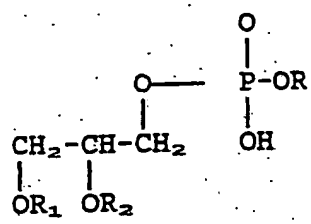
The data in the figures and the Table clearly illustrate that acylation of a drug containing a carboxy group to the hydroxy group of the glycerol backbone of a phospholipid significantly enhances the pharmokinetics of said drug. The phospholipid drug has an increased therapeutic index relative to the non-phospholipid drug. The phospholipid drugs prepared in accordance with the present invention can be more potent (Figure 1), less toxic (Figure 1), and more stable (Figure 8), and can have increased availability or distribution relative to the non-phospholipid drug. The enhanced pharmokinetics

of the phospholipid drugs prepared in accordance with the present invention makes it an extremely powerful weapon in the war against diseases.

The foregoing description of the invention has been presented for purposes of illustration and description and is not intended to be exhaustive or to limit the invention to the precise form disclosed. Other variations are possible in light of the teachings presented herein.

WHAT IS CLAIMED IS:

1. A compound of the formula



or pharmaceutically acceptable salts thereof wherein

one of R_1 and R_2 is a heteroatom fatty acid acyl group having 13-14 carbon atoms in the principal chain and up to a total of 18 carbon atoms, while the other is hydrogen, a heteroatom of fatty acid acyl group containing 13-14 carbon atoms in the principal chain and up to a total of 18 carbon atoms or an acyl group of a fatty acid containing 4-26 carbon atoms in the principal chain and up to a total of 30 carbon atoms and

R is a naturally occurring polar group characteristic of a glycerolphospholipid isolated from endogenous sources.

2. The compound according to Claim wherein R is

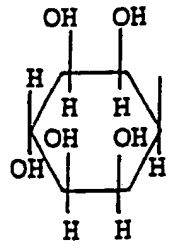
$-CH_2-CH_2-\overset{\oplus}{N}(CH_3)_3$;

$-H$;

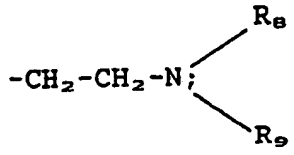
$-CH_2-CH(OH)-CH_2-OH$;

$-CH_2-CH(NH_2)-COOH$; or

$-CH_2-CH_2-NH_2$, or



, or



3. The compound according to any one of Claims 1-2 wherein the acyl group of a fatty acid contains 4-16 carbon atoms.

4. The compound according to any one of Claims 1-3 wherein R_1 is a heteroatom fatty acid acyl group and R_2 is hydrogen, a heteroatom fatty acid acyl group or an acyl group of a fatty acid.

5. The compound according to any one of Claims 1-4 wherein R_2 is a heteroatom fatty acid acyl group or hydrogen.

6. The compound according to any one of Claims 1-3 wherein R_2 is a heteroatom fatty acid acyl group and R_1 is hydrogen, a heteroatom fatty acid acyl group or an acyl group of a fatty acid containing 4-14 carbon atoms.

7. The compound according to any one of Claims 1-3 and 6 wherein R_1 is a heteroatom fatty acid acyl group, hydrogen, or an aryl group of a fatty acid containing 4-14 carbon atoms.

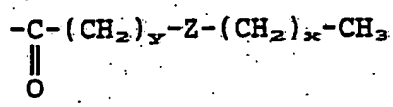
8. The compound according to any one of Claims 1-7 wherein R_1 and R_2 are both a heteroatom fatty acid acyl group.

9. The compound according to any one of Claims 1-8 wherein R_1 is the same as R_2.

10. The compound according to any one of Claims 1-3 wherein R_1 is a heteroatom fatty acid acyl group and R_2 is hydrogen or an acyl group of a fatty acid containing 4-14 carbon atoms.

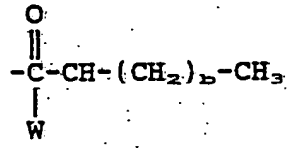
11. The compound according to any one of Claims 1-3 wherein R_2 is a heteroatom fatty acid acyl group and R_1 is hydrogen or an acyl group of a fatty acid containing 4-14 carbon atoms.

12. The compound according to any one of Claims 1-11 wherein the heteroatom fatty acid acyl group has the formula:



wherein x is 0-11 and y is 1-11 and x+y = 11 and Z is O or S.

13. The compound according to any one of Claims 1-11 wherein the heteroatom fatty acid acyl group has the formula:



wherein W is hydroxy, halo, lower alkoxy, mercapto or lower alkylthio; and

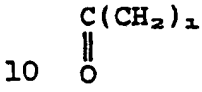
b is 11.

14. The compound according to Claim 13 wherein W is hydroxy or halo.

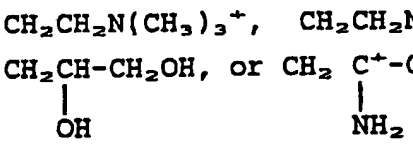
15. The compound according to Claim 14 wherein halo is Br or Cl.

16. The compound according to any one of Claims 1-12 wherein the heteroatom fatty acid acyl group is

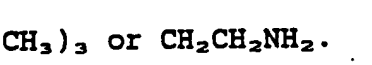
17. The compound according to any one of Claims 1-16 wherein the acyl group of the fatty acid is



18. The compound according to any one of Claims 1-17 wherein R is

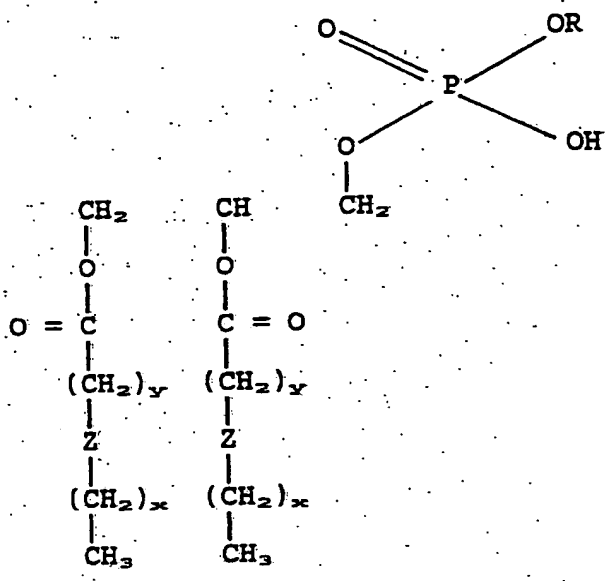


19. The compound according to any one of Claims 1-18 wherein R is



20. The compound according to any one of Claims 1-19 wherein Z is O.

21. The compound according to any one of Claims 1-20 having the formula:



or pharmaceutically acceptable salts thereof wherein

R is a naturally occurring polar head group characteristic of a glycerophospholipid isolated from endogenous sources;

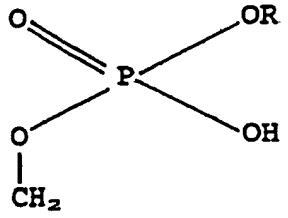
R_1 and R_2 are independently hydrogen or an alkyl fatty acid acyl group having 4-26 carbon atoms, and A is a heteroatom alkyl fatty acid acyl group having 3-25 carbon atoms

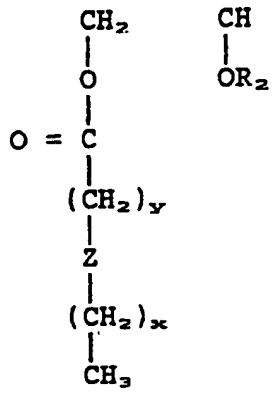
Z is O or S;

x = 0-11

y = 1-11 and x + y = 11.

22. The compound according to any one of Claims 1-20 having the formulae:





or pharmaceutically acceptable salts thereof wherein

R is a naturally occurring polar head group characteristic of a glycerophospholipid isolated from endogenous sources;

Z is O or S;

x = 0-11,

y = 1-11, and x + y = 11, and

R_1 and R_2 are independently hydrogen or  ;

and

R_7 is an alkyl group containing 3-18 carbon atoms.

23. The compound according to Claim 22 wherein R_7 is an alkyl group containing 13 carbon atoms.

24. The compound according to any one of Claims 21-23 wherein

R is

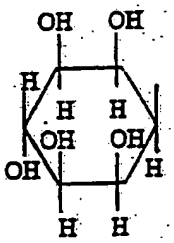
$-CH_2-CH_2-\overset{\oplus}{N}-(CH_3)_3$;

$-H$;

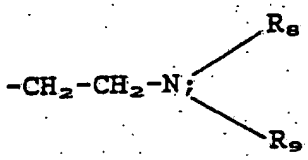
$-CH_2-\underset{\underset{OH}{|}}{CH}-CH_2-OH$;

$-CH_2-\underset{\underset{NH_2}{|}}{CH}-COOH$; or

$-CH_2-CH_2-NH_2$, or



, or

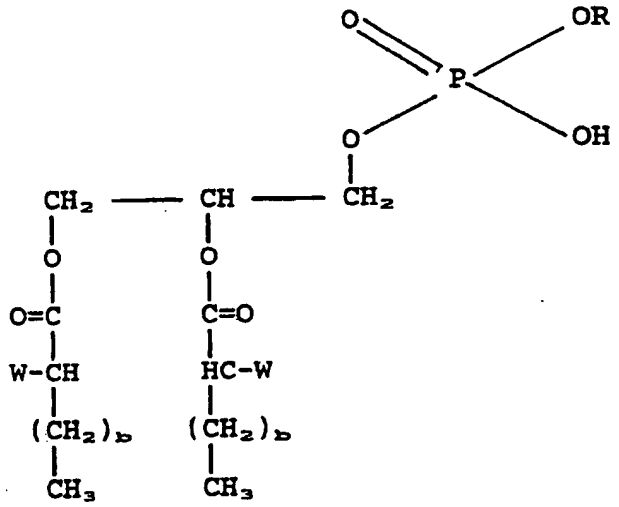


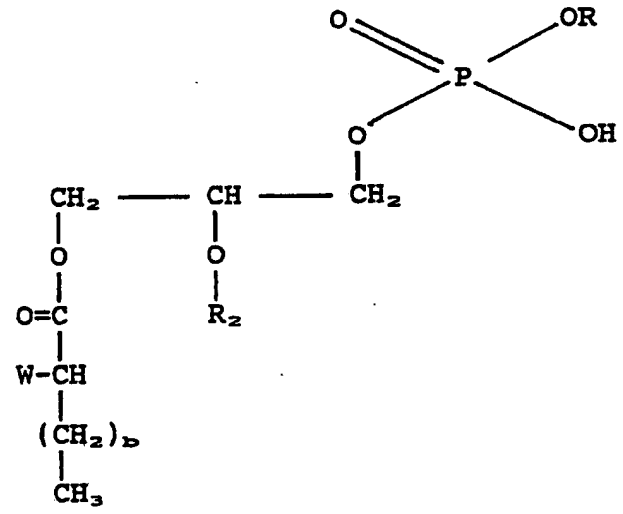
25. The compound according to any one of Claims 21-24 wherein R is $CH_2CH_2\overset{\oplus}{N}-(CH_3)_3$ or $CH_2CH_2NH_2$.

26. The compound according to any one of Claims 21-25 wherein Z is O.

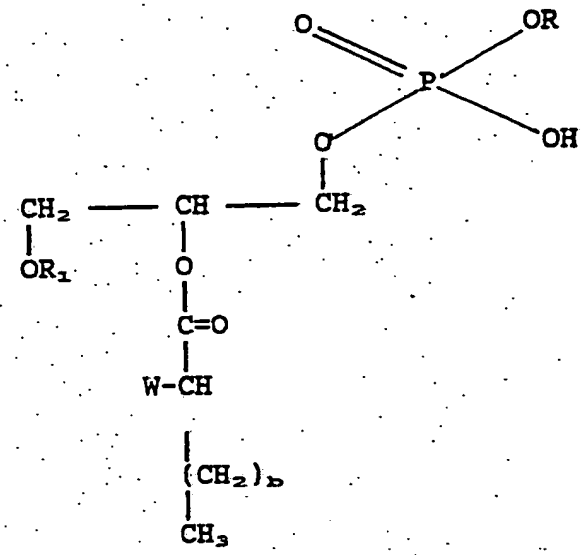
27. The compound according to any one of Claims 21-26 wherein x is 0 and y is 11.

28. The compound according to any one of Claims 1-9 having the formulae:





or



XII

or pharmaceutically acceptable salts thereof wherein

W is hydroxy, halo, lower alkoxy, mercapto or lower alkylthio,

b is 11

R is a naturally occurring polar head group characteristic of a glycerophospholipid isolated from endogenous sources;

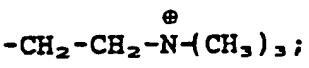
R_1 and R_2 are independently hydrogen or $C-R_7$, ‖ O

R_7 is an alkyl group containing 3-18 carbon atoms.

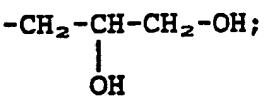
29. The compound according to Claim 28 wherein W is hydroxy or halo.

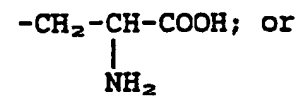
30. The compound according to Claim 29 wherein halo is Br or Cl.

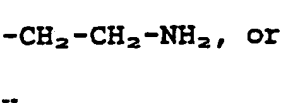
31. The compound according to any one of Claims 28-30 wherein R is

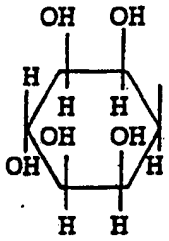


-H;

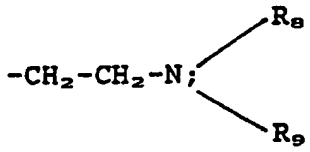




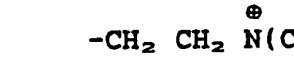


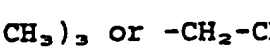


, or



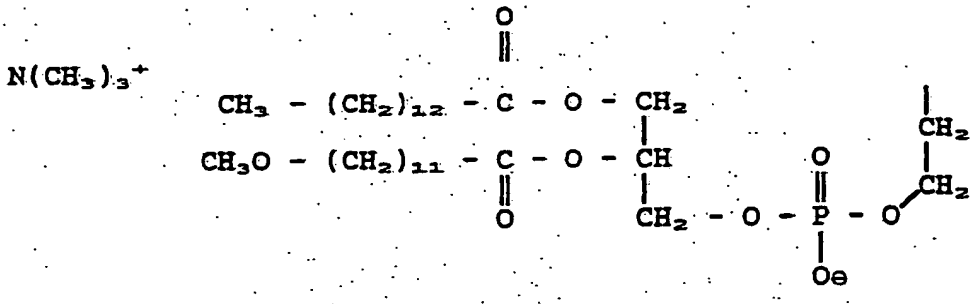
32. The compound according to any one of Claims 28-31 wherein R is



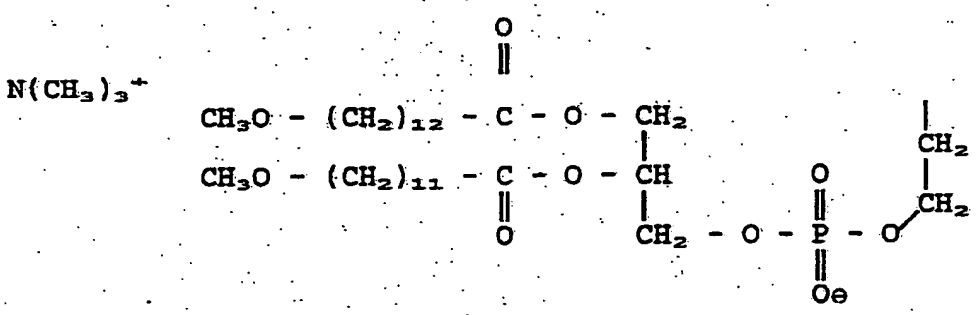
or  .

33. The compound according to any one of Claims 28-32 wherein R_7 is an alkyl group containing 3-7 or 13 carbon atoms.

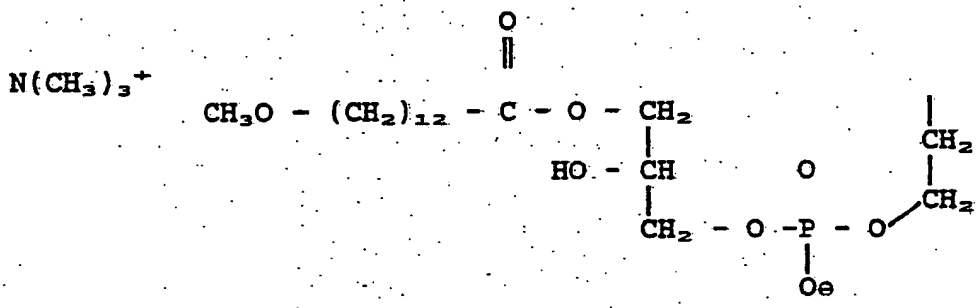
34. The compound according to Claim 1 of the formula:



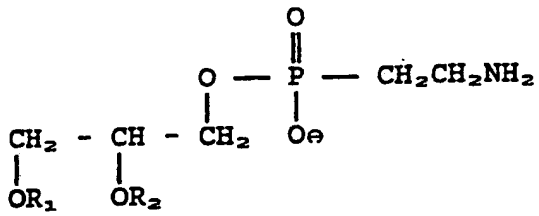
35. The compound according to Claim 1 of the formula:



36. The compound of Claim 1 of the formula:



37. The compound according to Claim 1 which is



wherein

R_1 is myristoyl or 12-methoxydodecanoyl and

R_2 is 12-methoxydodecanoyl acid.

38. The compound according to any one of Claims 1-37 wherein the configuration at the carbon in the Sn-2 position is L.

39. The compound according to any one of Claims 1-37 wherein the configuration at the carbon in the Sn-2 position is D.

40. A substantially pure compound of any of Claims 1-39.

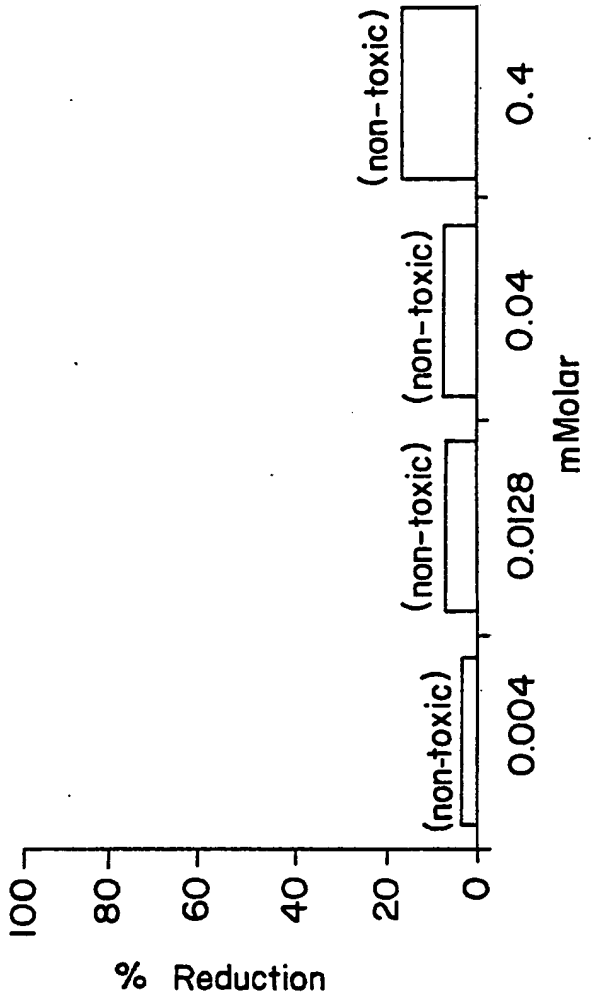
41. A method for the inhibition of protein myristoylation in animals comprising administering to said animal an effective amount of a compound according to any one of claims 1-40.

42. A method for the inhibition of retroviral proliferation in an animal comprising administering to said animal a retroviral proliferation inhibiting amount of the compound according to any one of Claims 1-40.

43. A method for the treatment of AIDS in an animal which comprises administering to said animal an anti-AIDS effective amount of the compound according to any of Claims 1-40.

44. A pharmaceutical composition suitable for administration to an animal in need thereof comprising a pharmaceutically acceptable carrier and an effective amount of a compound according to any one of Claims 1-40.

FIG. 1a


100
80
60
40
20
0
% Reduction
(non-toxic)
(non-toxic)
(non-toxic)
(non-toxic)
0.004
0.0128
0.04
0.4
mMolar

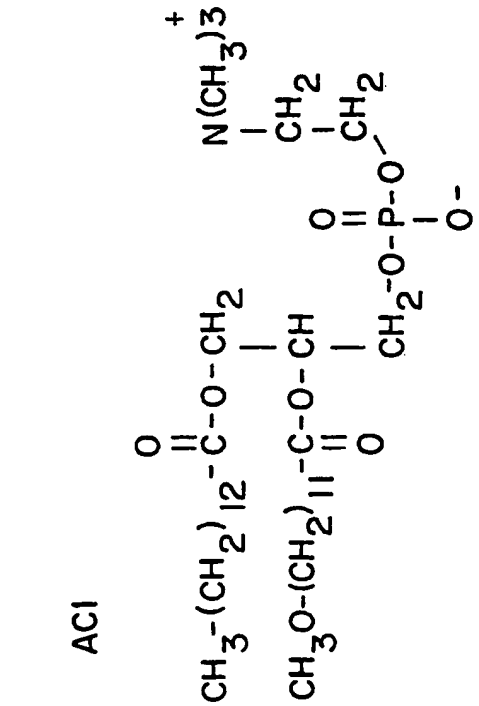
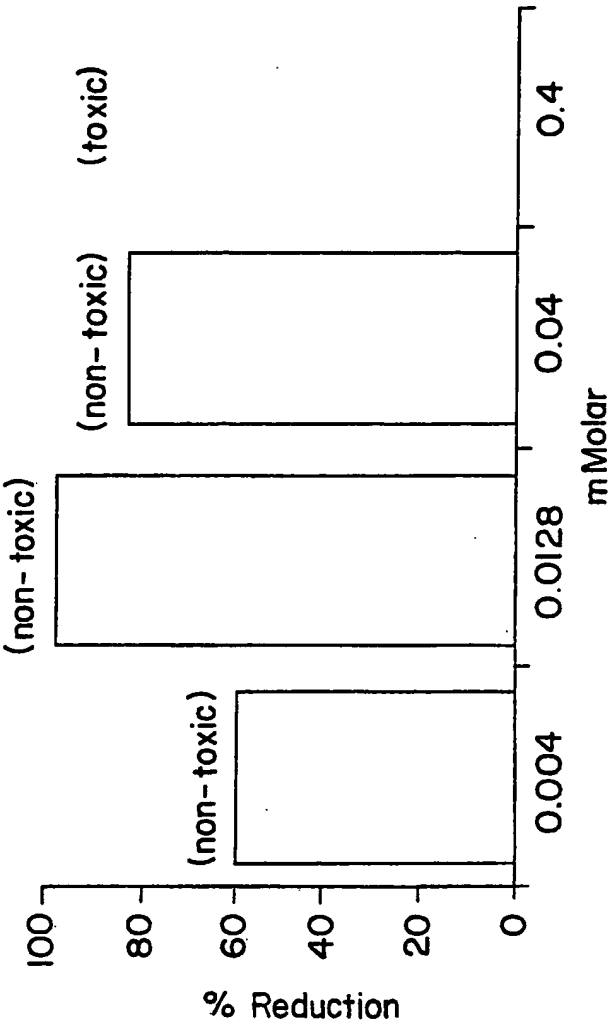

ACI
$CH_3-(CH_2)_{12}-C(=O)-O-CH_2$
$CH_3O-(CH_2)_{11}-C(=O)-O-CH$
$CH_2-O-P(=O)(-O^-)-O-CH_2$
$N^+(CH_3)_3$

FIG. 1b



AC2

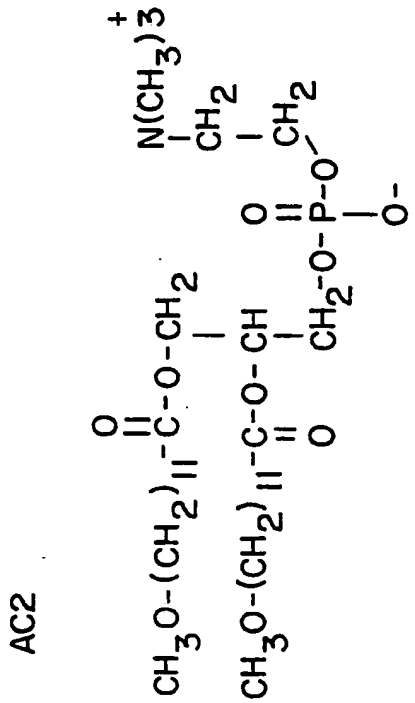
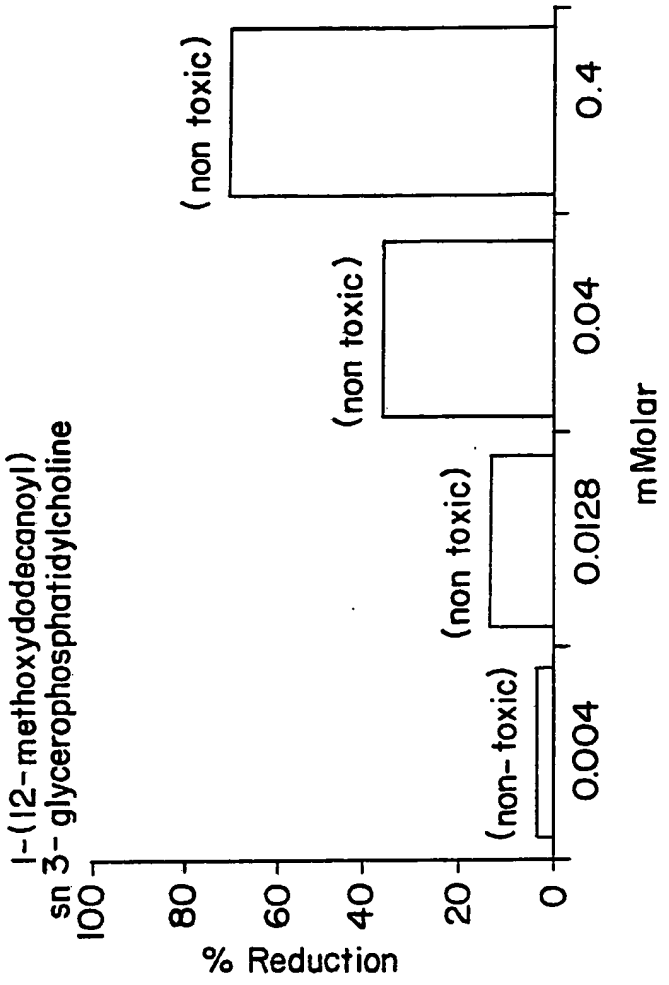


FIG. 1c


1-(12-methoxydodecanoyl)
sn 3- glycerophosphatidylcholine
100
80
60
40
20
0
% Reduction
(non toxic)
(non toxic)
(non toxic)
(non-toxic)
0.4
0.04
0.0128
0.004
mMolar

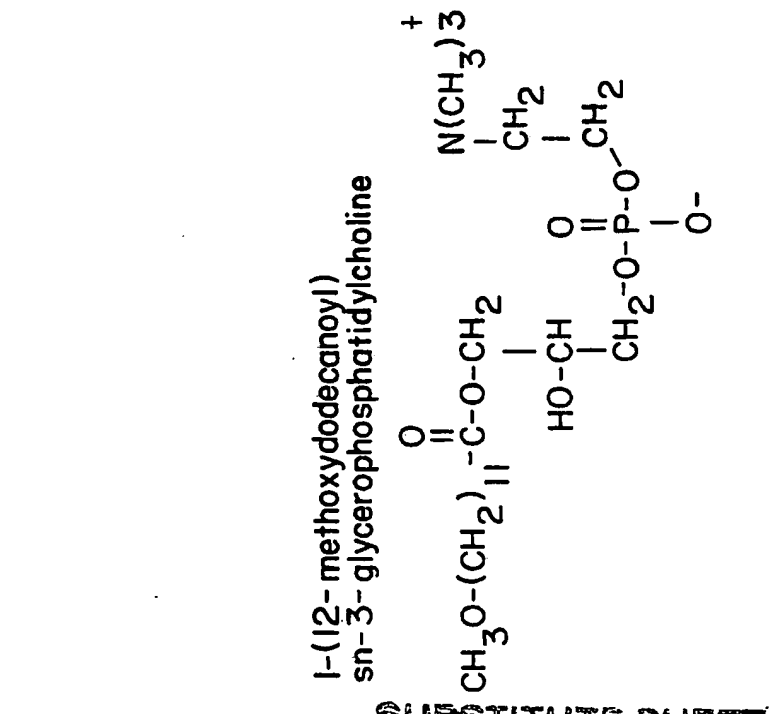
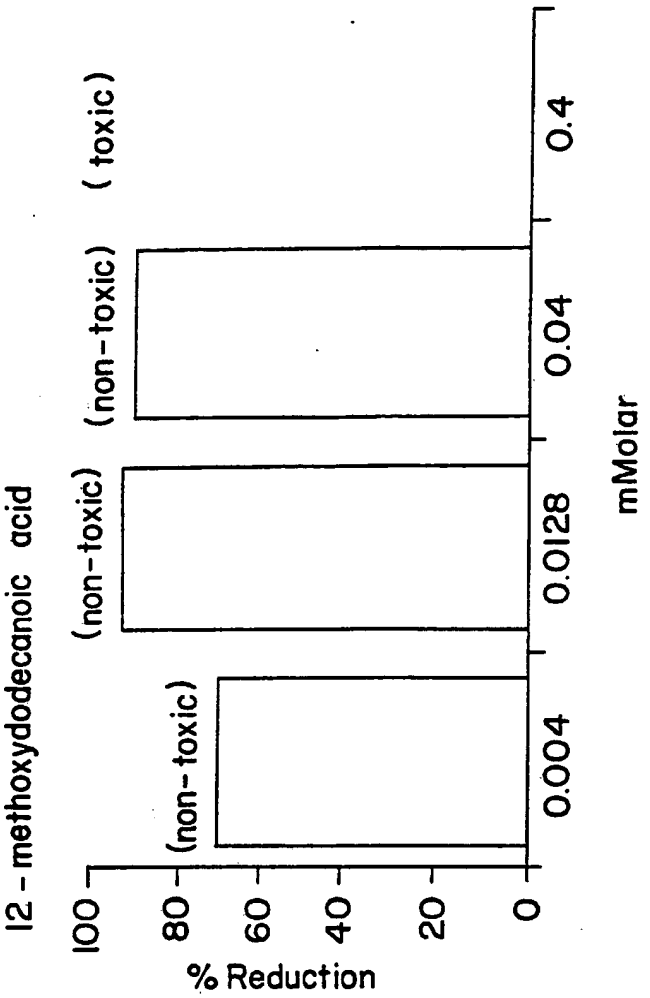

1-(12-methoxydodecanoyl)
sn-3-glycerophosphatidylcholine
CH3O-(CH2)11-C-O-CH2
HO-CH
CH2-O-P-O-CH2
N(CH3)3+

FIG. Id



12-methoxydodecanoic acid

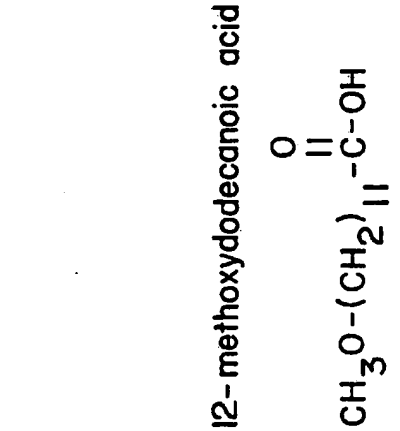


FIG. 2A

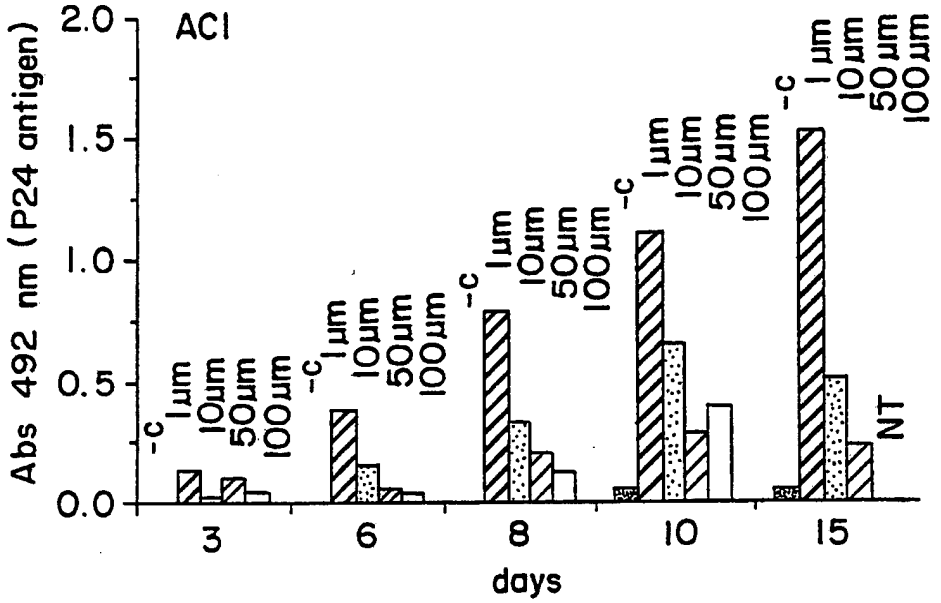

AC1
Abs 492 nm (P24 antigen)
2.0
1.5
1.0
0.5
0.0
-c
1 µm
10 µm
50 µm
100 µm
NT
3
6
8
10
15
days

FIG. 2B

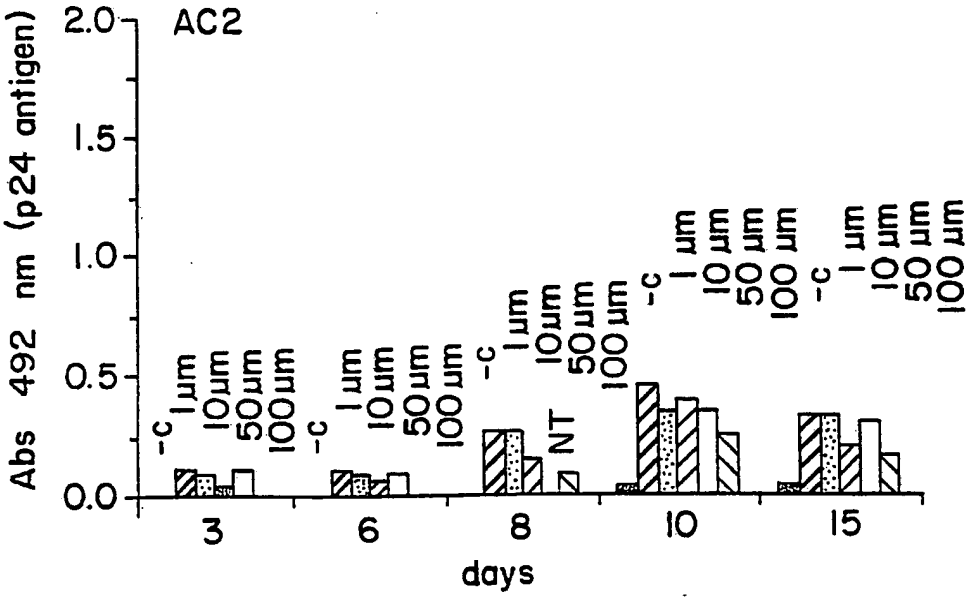

AC2
Abs 492 nm (p24 antigen)
2.0
1.5
1.0
0.5
0.0
-c
1 µm
10 µm
50 µm
100 µm
NT
3
6
8
10
15
days

FIG. 2C

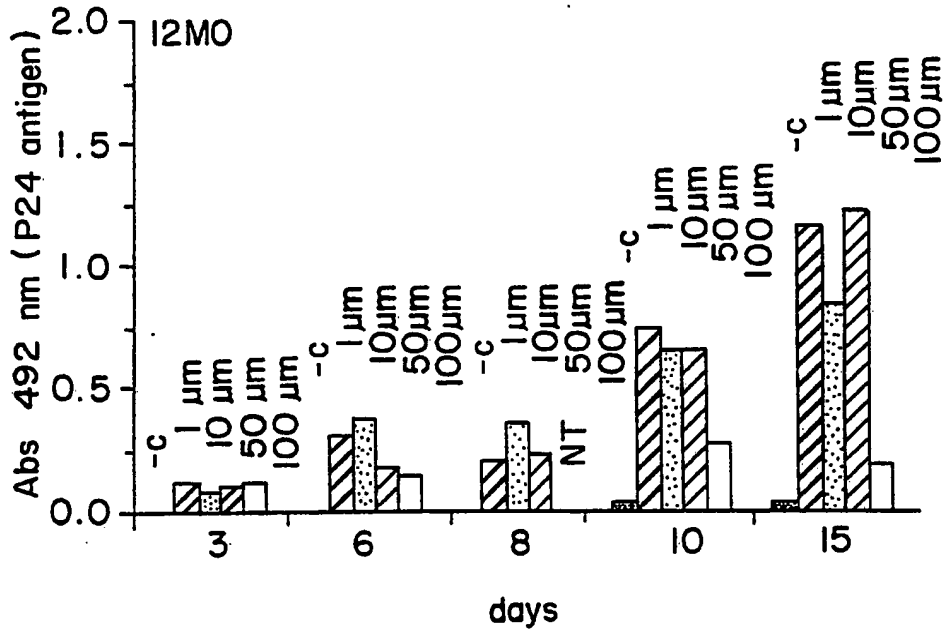

12MO
Abs 492 nm (P24 antigen)
2.0
1.5
1.0
0.5
0.0
-c
1 µm
10 µm
50 µm
100 µm
NT
3
6
8
10
15
days

FIG. 3A

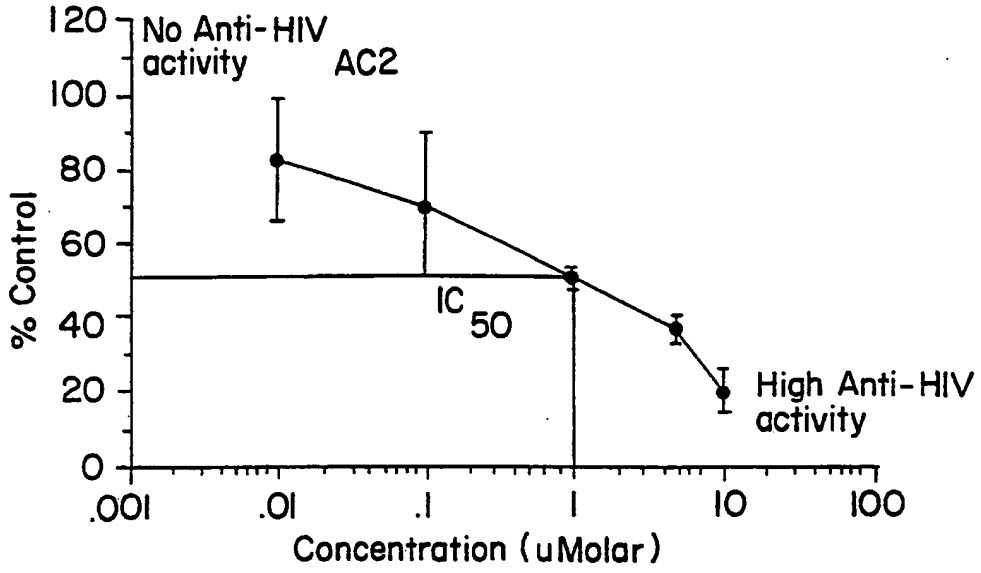

120
100
80
60
40
20
0
% Control
No Anti-HIV activity
AC2
IC 50
High Anti-HIV activity
.001
.01
.1
1
10
100
Concentration (uMolar)

FIG. 3B

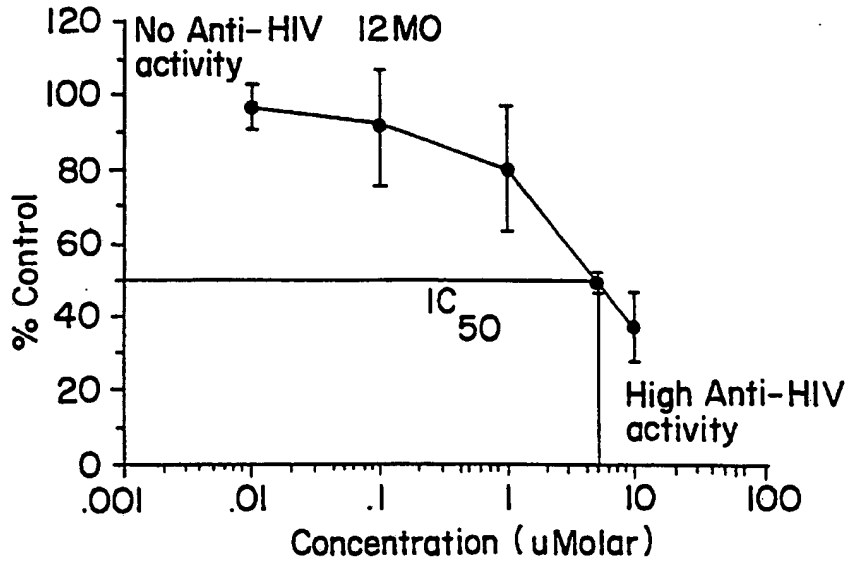

120
100
80
60
40
20
0
% Control
No Anti-HIV activity
12MO
IC 50
High Anti-HIV activity
.001
.01
.1
1
10
100
Concentration (uMolar)

FIG. 4A

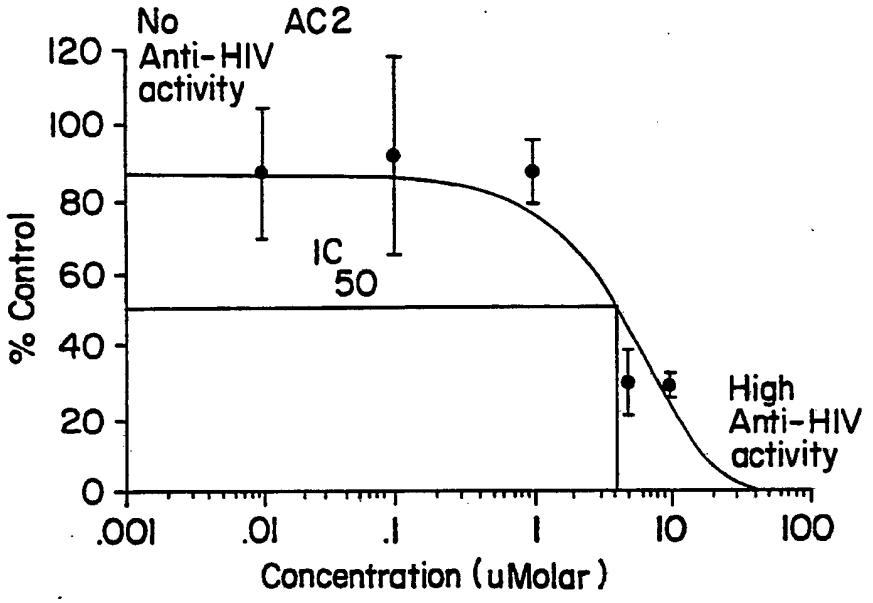

No
Anti-HIV
activity
AC2
120
100
80
60
40
20
0
% Control
IC
50
High
Anti-HIV
activity
.001
.01
.1
1
10
100
Concentration (uMolar)

FIG. 4B

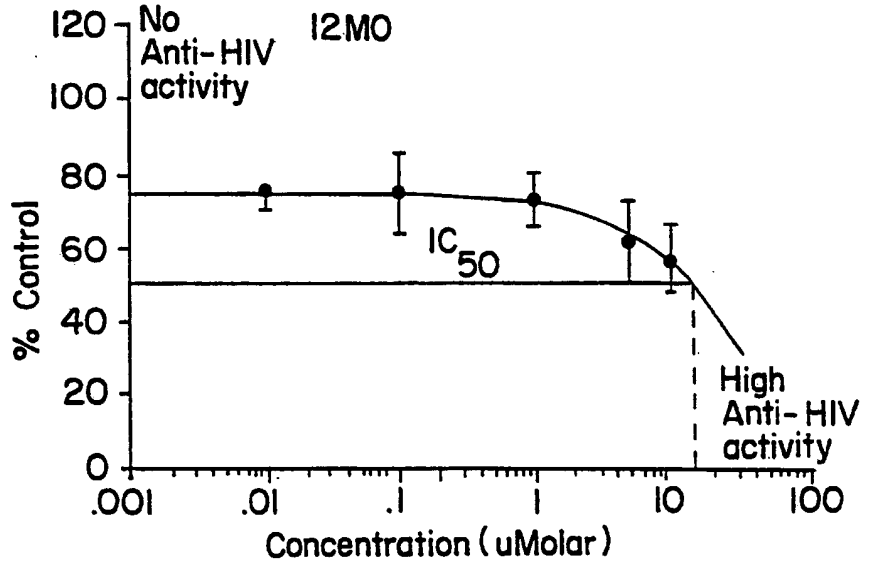

No
Anti-HIV
activity
12MO
120
100
80
60
40
20
0
% Control
IC50
High
Anti-HIV
activity
.001
.01
.1
1
10
100
Concentration (uMolar)

FIG. 5A

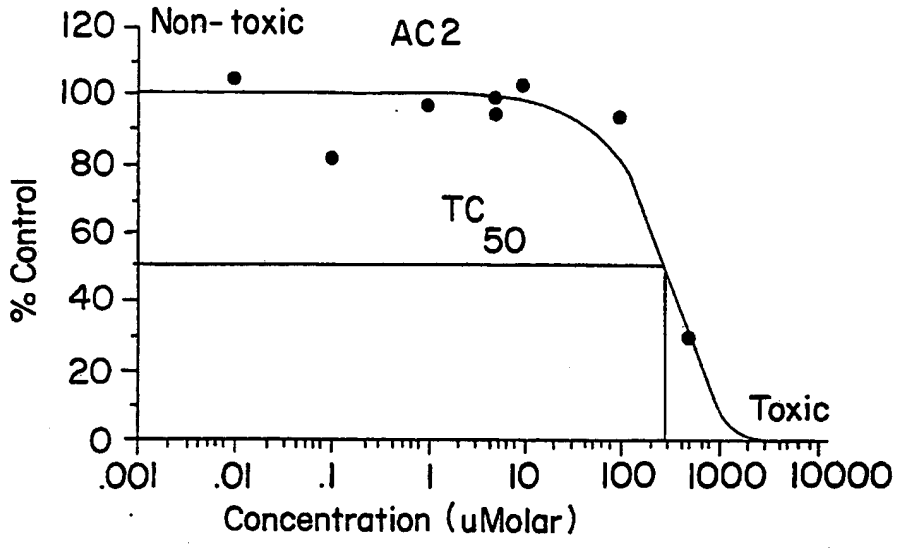

120
100
80
60
40
20
0
% Control
Non-toxic
AC2
TC 50
Toxic
.001
.01
.1
1
10
100
1000
10000
Concentration (uMolar)

FIG. 5B

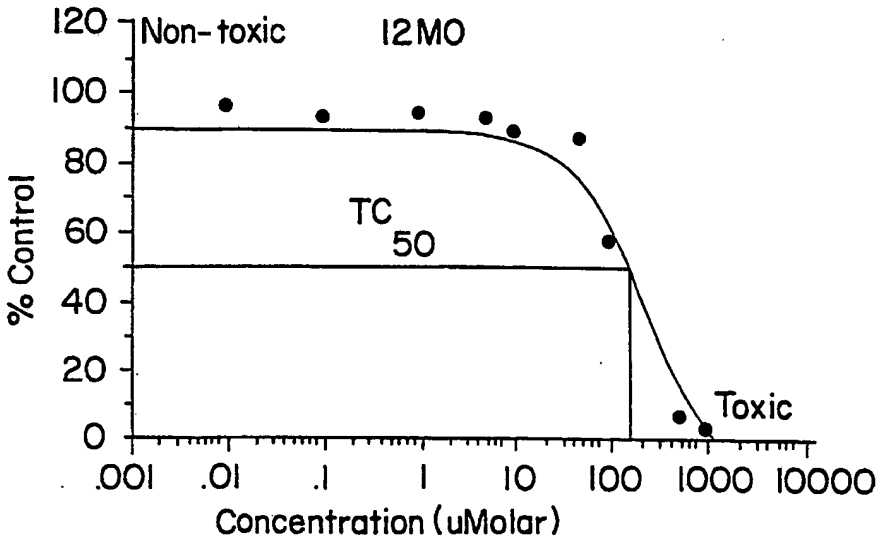

120
100
80
60
40
20
0
% Control
Non-toxic
12MO
TC 50
Toxic
.001
.01
.1
1
10
100
1000
10000
Concentration (uMolar)

FIG. 6

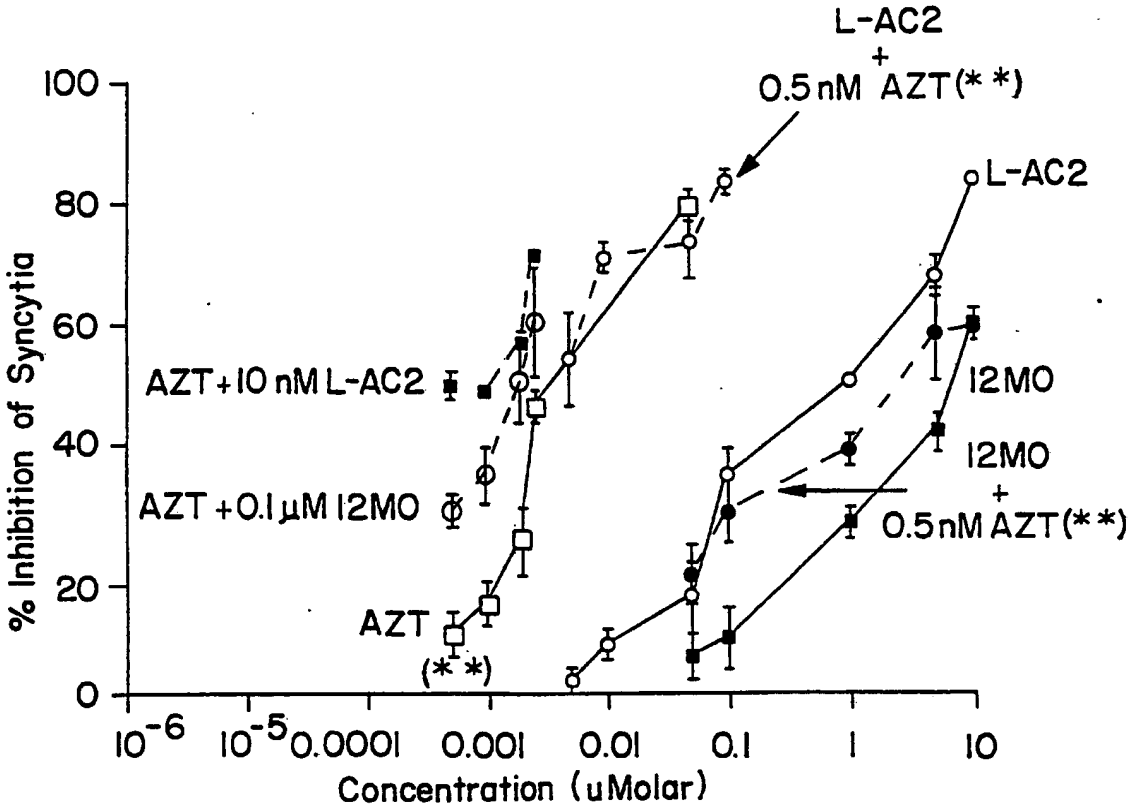

L-AC2
+
0.5 nM AZT(**)
L-AC2
AZT+10 nM L-AC2
AZT +0.1 μM I2MO
I2MO
I2MO
+
0.5nM AZT(**)
AZT
(**)
% Inhibition of Syncytia
100
80
60
40
20
0
10^-6
10^-5
0.0001
0.001
0.01
0.1
1
10
Concentration (uMolar)

FIG. 7

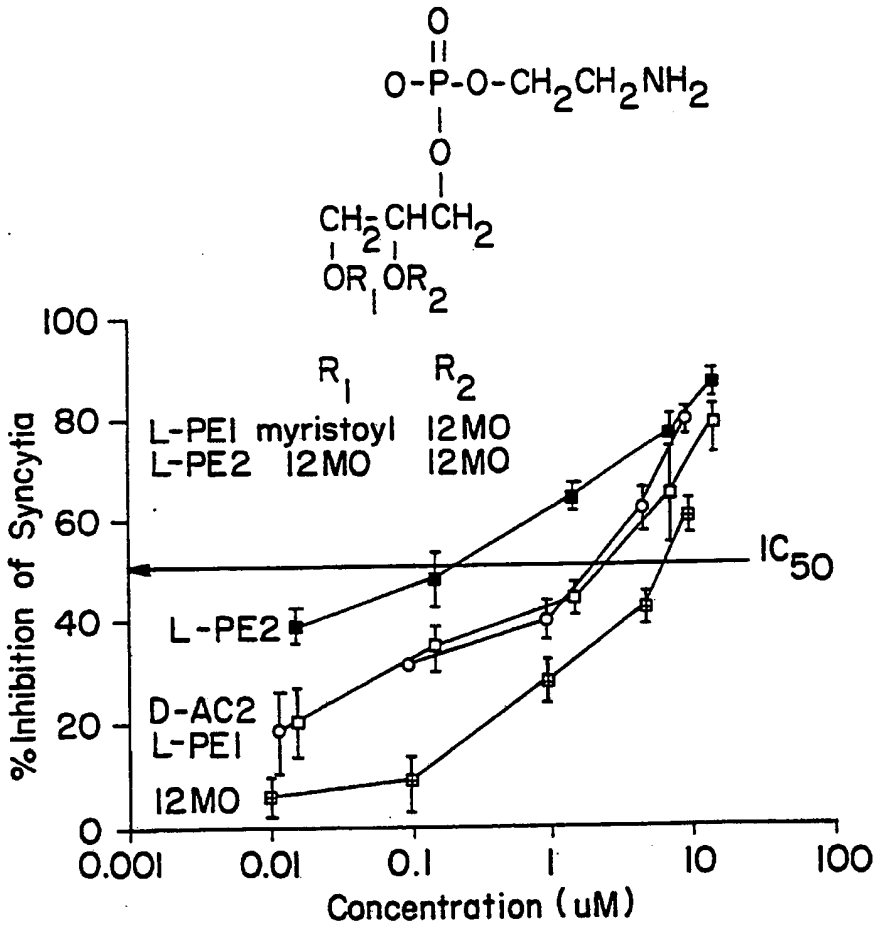
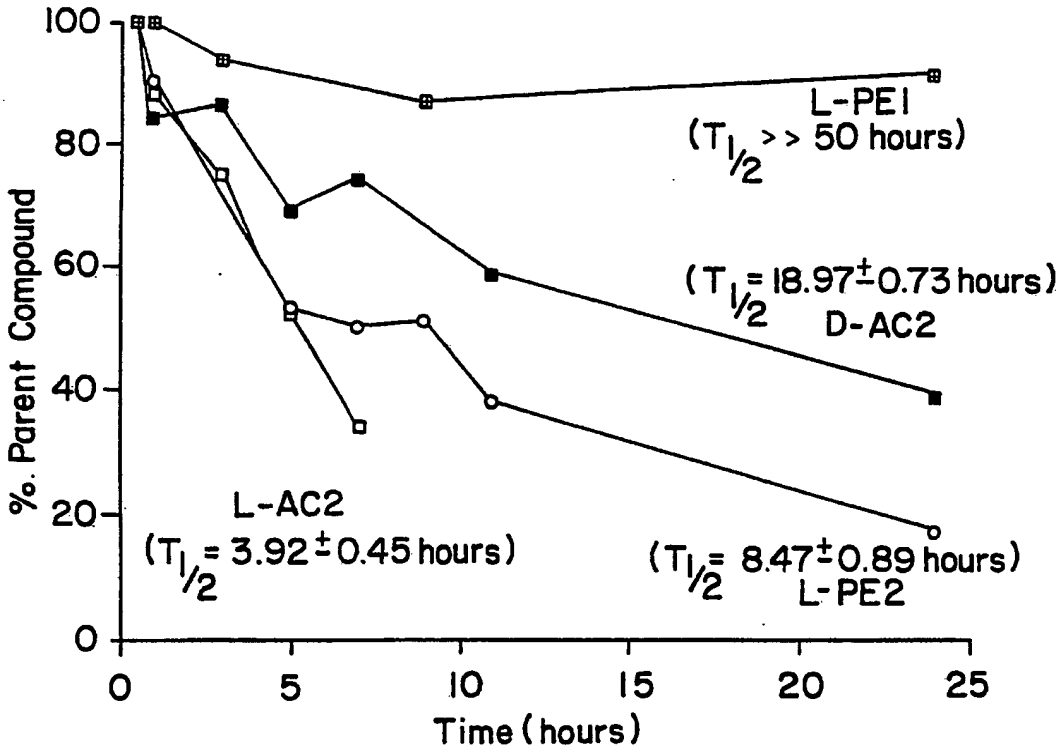

O
O-P-O-CH2CH2NH2
O
CH2 CHCH2
OR1 OR2
R1 R2
L-PE1 myristoyl 12MO
L-PE2 12MO 12MO
IC50
L-PE2
D-AC2
L-PE1
12MO
% Inhibition of Syncytia
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Concentration (uM)

FIG. 8


100
80
60
40
20
0
% Parent Compound
0
5
10
15
20
25
Time (hours)
L-PE1
($T_{1/2}$ >> 50 hours)
($T_{1/2}$ = 18.97±0.73 hours)
D-AC2
L-AC2
($T_{1/2}$ = 3.92±0.45 hours)
($T_{1/2}$ = 8.47±0.89 hours)
L-PE2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/03650

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07F9/10; A61K31/66; C07F9/117

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07F ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0 415 902 (WASHINGTON UNIVERSITY) 6 March 1991 cited in the application see the whole document ---	1,41-44
A	EP,A,0 316 117 (SCIENSCOPE INTERNATIONAL N.V.) 17 May 1989 see the whole document -----	1,41-44

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18 JUNE 1993	- 5. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	BESLIER L.M.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9303650 SA 73327

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 18/06/93
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0415902	06-03-91	US-A-	5073571	17-12-91
		CA-A-	2024489	02-03-91
		JP-A-	3109322	09-05-91
EP-A-0316117	17-05-89	AU-A-	2619988	01-06-89
		JP-T-	2502096	12-07-90
		WO-A-	8904314	18-05-89